Iranian Journal of Medical Physics

ijmp.mums.ac.ir

Expression of DDB2, XPC, and GADD45 Genes after Whole Body Gamma Irradiation

Reza Fardid[1,2], Manouchehr Ghasemi[1], Maliheh Rostamyari[1*], Gholamhassan Haddadi[1,2], Mohammad Ali Takhshid[3], Abbas Behzad Behbahani[3]

1. Department of Radiology, Faculty of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
2. Ionizing and Non-Ionizing Radiation Protection Research Center (INIRPRC), Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Laboratory Medicine, School of Paramedical Sciences, Shiraz University of Medical Sciences, Shiraz, Iran

ARTICLE INFO

Article type:
Original Paper

Article history:
Received: Aug 01, 2021
Accepted: Nov 04, 2021

Keywords:
Gamma Radiation
Rats
Gene Expression
Lymphocytes
DNA Damage

ABSTRACT

Introduction: The stimulation of DNA repair mechanisms is an immediate response to radiation-induced damage. Monitoring the expression of DNA-repair-related genes would be a beneficial method to identify bio-dosimeter of radiation exposure, particularly for challenging low-dose radiation. In this study, we aimed to evaluate the effect of different low doses of gamma radiation on the expression of DDB2, XPC, and GADD45A genes involved in DNA-damage repair mechanisms.

Material and Methods: Forty-eight male rats were divided into a control group and five exposure groups. The latter groups exposed to various doses of γ-rays (Co-60) ranged from 20 mGy to 1000 mGy. 24 h after irradiation, isolated lymphocytes from collected blood samples were used for evaluating gene expression levels by real-time quantitative polymerase chain reaction (qRT-PCR). Data were expressed as means ± SD and were statistically evaluated using one-way ANOVA or Kruskal-Wallis test. P value<0.05 was considered as a significant value.

Results: DDB2, GADD45A, and XPC expression remained unchanged at a dose of 20 mGy, and at doses above 20 mGy, they changed significantly. XPC and GADD45A altered significantly at 50 mGy while DDB2 changed significantly after exposure to 100, 500, and 1000 mGy.

Conclusion: Low doses of gamma radiation (less than 1 Gy) can significantly affect DDB2, XPC, and GADD45A expression, three central genes in the DNA-damage repair process. The extent of the gene expression changes at higher doses of 100, 500, and 1000 mGy seems more severe than that of their lower counterparts (50 mGy).

► *Please cite this article as:*
Fardid R, Ghasemi M, Rostamyari M, Haddadi Gh, Takhshid MA, Behzad Behbahani A. Expression of DDB2, XPC, and GADD45 Genes after Whole Body Gamma Irradiation. Iran J Med Phys 2022; 19: 207-213. 10.22038/IJMP.2021.59000.1990.

Introduction

In the modern world, exposure to ionizing radiation has become an inseparable part of our routine life. From the members of the public to occupationally-exposed individuals, all of us are exposed to ionizing radiation [1]. Ionizing radiation causes considerable damage to organ-forming cells, including single-stranded and double-stranded DNA breaks, DNA base damage, base mismatches, the formation of purine and pyrimidine loci, telomere dysfunction, and DNA-protein cross-links [2]. Moreover, ionizing radiation (IR) can cause oxidative stress by forming reactive oxygen species (ROS) [3, 4]. It has been estimated that the number of daily DNA-damaging due to oxidative stress is about 2×10^4 per cell of the human body [1]. Responding to these damages, various repair mechanisms and different signaling cascades exist, which trigger and change gene expression levels, leading to the activation of different proteins [5, 6]. Each of these repair pathways is pertinent to a specific type of DNA lesions. The most common types of DNA-repair mechanisms and a brief schematic of the DNA repair pathway associated with the expression of DDB2, XPC, and GADD45 genes have been summarized in figure1. Base excision repair (BER) acts as a guard against damage caused through cellular metabolisms such as altered base, abasic site, and single-strand breaks. During BER, various DNA glycosylases and APE1 endonuclease removed the damaged base. The essential repair mechanism for bulky DNA lesions is nucleotide excision repair (NER), which removes the DNA helix distortions caused by exogenous or endogenous agents. Mismatch repair (MMR) can remove base mismatches due to replication-related errors. Repairing a most genotoxic type of DNA damage, double-strand breaks (DBS), two mechanisms have evolved, homologous recombination (HR) and non-homologous end-joining [7, 8].

*Corresponding Author: Tel: +9871 32295023; Email: maliheh.rostamyari@gmail.com

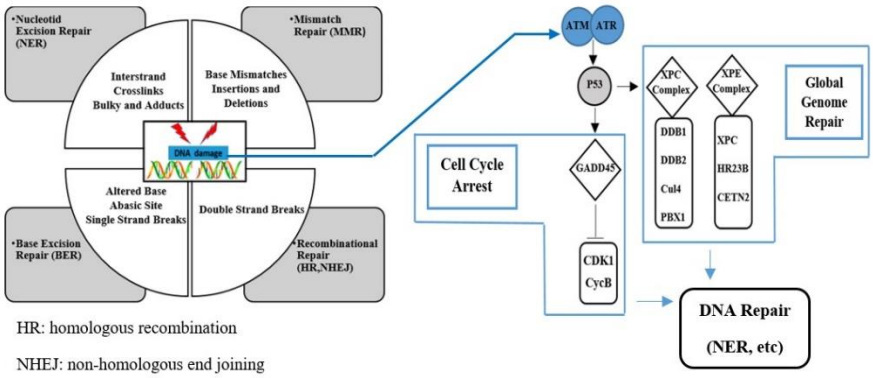

Figure 1. The most common types of DNA damages that can be caused by irradiation and their distinct DNA repair mechanisms. In response to DNA damage, ATM and/or ATR prompt the activation of P53, which in turn activates a cascade of genes and proteins that contribute to DNA repair processes. These genes and proteins are activated via global genome repair and cell cycle arrest or delay.

The instability of gene and protein expression in post-irradiated cells can be used as an appropriate diagnostic tool for elucidating the effect of irradiation on cells [9]. Radiation-induced DNA damage depends on different factors such as the type of radiation, the linear energy transfer (LET), and the dose rate [10-12]. Numerous evidence indicated that exposure to low-dose ionizing radiation from natural sources (cosmic radiation, terrestrial radiation, primordial radionuclides, etc.), artificial and human-made sources (nuclear power plants, industrial sources, medical procedure, nuclear fuel cycle, radioactive waste, etc.) are more common than high-dose radiation [13]. Although the linear non-threshold model (LNT) is used to estimate possible risks from exposure to a low dose, this model's validity is a controversial issue since the non-targeted effect of ionizing radiation has been observed significantly at low dose. These observations indicate that living organisms' response to low dose radiation differs from that of high dose radiation and requires further investigation [13, 14]. Many studies have shown that genetic and epigenetic factors may influence the non-targeted effects of radiation. On the other hand, genetic changes, accumulated mutations, and chromosomal aberration, which are all critical features in cancer development, require DNA damage or an incorrect DNA repair process to occur [15, 16].

The process of DNA repair plays a vital role in protecting individuals against the side effects of ionizing radiation, including cancer initiation. The DNA repair process is controlled by a set of genes encoding enzymes responsible for catalyzing the cell's response to DNA damage. Unrepaired DNA function or failure in DNA repair control by the cell cycle can have severe consequences for the cell [17, 18]. The DNA repair process's importance in patients with defects in the DNA repair system is more elucidated since they are more potentiated to DNA damage-induced carcinogenesis [19]. Based on reliable evidence, the expression of many genes involved in cellular DNA damage and repair functions may be altered due to ionizing radiation [20]. Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-Kβ) and tumor protein P53 are among the most prime transcription factors which are activated in response to IR-induced DNA damage [21-24]. TP53 acts as a tumor suppressor and enhances the expression of many genes associated with cell cycle arrest, apoptosis, autophagy, and senescence [22, 23]. The stimulation of DNA repair mechanisms is an immediate response to IR-induced damage. It seems, then, the monitoring of the expression of DNA-repair related genes would be a beneficial method to identify bio-dosimeter of exposure to radiation, particularly for a challenging low dose radiation. Therefore, the present study aimed to evaluate the effect of different low-doses of gamma radiation on the expression of three prime genes, DDB2, XPC, and GADD45A, involved in nucleotide excision repair (NER), base excision repair (BER), and cell cycle regulation.

Materials and Methods

In this experiment, male Sprague-Dawley rats were bought from the Center of Comparative and Experimental Medicine at the Shiraz University of Medical Science in Shiraz, Iran. The study was approved by the Shiraz University of Medical Sciences ethics committee in Iran, and all participants signed a consent form under local ethical guidelines (Approva No.90036). Before the experiment began, all the animals were kept under standard controlled conditions: a 12h light/12h dark cycle, a temperature of 23 2 ºC, and a humidity of 55 5%. They were fed a standard pellet diet, and they could drink as much water as they wanted. The experiment was done on 48 male rats that weighed between 200 and 250 g. The rats were randomly split into a control group that wasn't exposed to radiation and five exposure groups that were (8 rats in each group). The rats in the exposure groups were transferred to a cobalt 60-gamma irradiator (Theratron 780, Atomic energy of Canada limited, Canada) facility in the radiotherapy department, Namazi Hospital, Shiraz, Iran. After putting the rats under anesthesia, they were confined in acrylic restrainers with adequate ventilation,

and then they were subjected to varying dosages of whole-body gamma radiation (20, 50, 100, 500, and 1000 mGy; 60CO gamma rays; 36.32 cGy/min; SSD = 80 cm; fixed field size of 30 × 30 cm2). Twenty-four hours after the animals were exposed to gamma irradiation, blood samples were obtained from the heart puncture of the anesthetized animals and placed in sterile Ethylenediaminetetraacetic acid (EDTA) tubes before being transported to the laboratory for the next step in the process.

Lymphocyte Isolation

In accordance with the established procedure, lymphocytes were isolated from each blood sample by using ficoll Lymphodex (Innotrain, Germany). Phosphate-buffered saline (PBS) was used to dilute the blood by a factor of three before it was layered over the ficoll at a ratio of blood plus PBS to ficoll of 2:1. After that, the blood was centrifuged for twenty minutes at a temperature of room temperature and 3,000 revolutions per minute. The lymphocyte layer was separated and, after three times washing with PBS and was centrifuged at 1400 rpm for 10 min. The supernatant layer was removed, and 500 µl of PBS was added to the sediment (lymphocytes layer) and was centrifuged at 1600 rpm for 5 min. An isolated lymphocyte was then used for RNA extraction.

Quantitative real-time RT-PCR (QPCR)

The RNX-Plus Kit's instructions were followed to carry out the RNA extraction procedure. After the RNA was extracted, it was analyzed with an agarose gel electrophoresis and a nanodrop® spectrophotometer (both manufactured by Thermo Fisher Scientific, LNC) to determine its concentration, purity, and overall integrity. With the Thermo Scientific kit (Thermo Science, USA), 1 µg total RNA was DNase I treated, and EDTA inactivated. It was then kept at -80 ˚C until cDNA was synthesized. Reverse transcription of treated RNAs into cDNA utilizing Suprime Script RTase, Oligo-dT, and dNTPs (Thermo scientific). The Ampliqon Taq DNA polymerase Master Mix RED kit (Denmark) was used to confirm the fidelity of produced cDNA. This reaction employed glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers, and the end products were put on a 2% agarose gel. Table 1 summarizes polymerase chain reaction cycling conditions. Finally, RT PCR was done using SYBR Green Real-Time PCR Kit (Yekta Tajhiz, Iran) and 48-well Step One TM ABI plates. Table 2 lists the specific primer sequences. The Ct number of all genes normalized to GAPDH in each sample and relative changes for mRNA were calculated based on the ΔΔCt method[25].

Table 1. Thermal cycling conditions of polymerase chain reaction

Step	Number of cycle	Temperature (˚C)	Time (min)
Initial denaturation	1	95	2
Denaturation	40	95	0.5
Annealing	40	58-64	0.5
Extension	40	72	0.5
Final extension	1	72	5

Table 2. The List of Primer Sequences and their Product Size Used for Real-Time PCR Analysis

Primer name	Sequence (5´- 3´)	Length (bp)
DDB2 (forward)	AAGTTGGGCAAAGCCACCTG	132
DDB2 (reverse)	GTGCCATGCCAAGGACGTTG	
GADD45A (forward)	CCTGCACTGTGTGCTGGTGA	106
GADD45A (reverse)	CCACTGATCCATGTAGCGACTTTC	
XPC (forward)	GTGGACCAAGGCACCGATGAAG	119
XPC (reverse)	ACAGGCAGGTCAGACCGCGAG	

Statistical analysis

The data were analyzed using Graph Pad Prism 8.0. The Shapiro-Wilk test was used to assess the data's normality. The differences in gene expression profiles were also analyzed using one-way ANOVA or Kruskal-Wallis tests. All results were shown as mean±SD of at least three independent experiments run in duplicate, and a P value<0.05 was considered as a significant value.

Results

Expression of DDB2 gene after exposure to different low-doses of gamma ray:

As can be seen in Figure2, the expression of DDB2 due to radiation, almost decreased significantly in all doses of radiation except at 20 mGy. Besides, its reduction at a dose of 50 mGy was not statistically different from that of the control group. A significant difference was observed between DDB2 expression in low doses of 20 and 50 mGy and its expression in other groups, including 100, 500, and 1000 mGy.

Expression of GADD45A gene after exposure to different low-doses of gamma ray:

Although radiation resulted in the upregulation of GADD45A at all given doses of gamma radiation, it was statistically significant just at doses of 50 and 1000 mGy. According to data obtained from multiple comparison analysis, there was a significant change between various groups, illustrated in figure3.

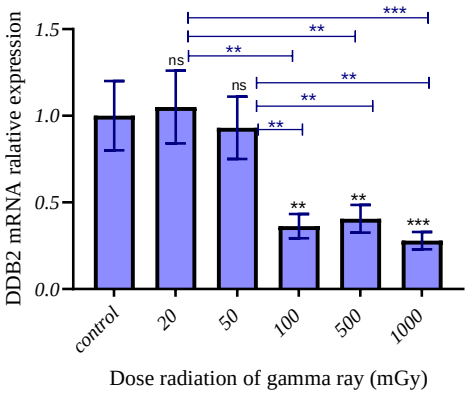

Figure 2. Effect of low-dose gamma exposure on DDB2 gene expression in whole-body rat's peripheral lymphocytes 24 hours after irradiation. (Error bars indicated SD, **P<0.01, ***P<0.001, and ns= Non-significant)

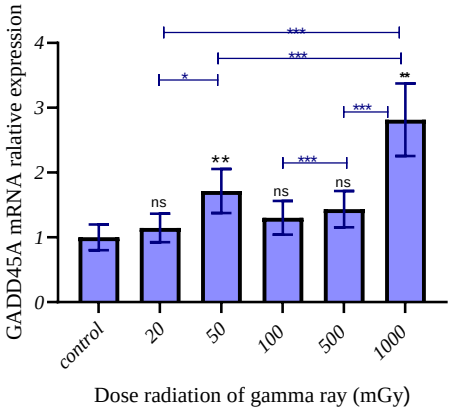

Figure 3. Effect of low-dose of gamma exposure on GADD45A gene expression in whole-body rat's peripheral lymphocytes 24 hours after irradiation. (Error bars indicated SD, *P<0.05, **P<0.01, ***P<0.001, and ns= Non-significant)

Expression of XPC gene after exposure to different low-doses of gamma ray:

According to the data shown in Figure4, radiation has induced an increase in XPC expression at doses of 20 and 50 mGy; however, it was not significant at 20 mGy. Furthermore, the XPC gene was downregulated non-significantly at doses above 50mGy compared to the control group. In addition, multiple compression between irradiated groups shows a significant difference between the expression of XPC at a dose of 20 mGy with that of 500 (P<0.01) and 1000 (P<0.05) mGy. Moreover, the expression of XPC, after exposing to 50 mGy, differed from that in doses above 50 mGy (including 100 (P<0.05), 500 (P<0.001), and 1000 mGy (P<0.01)).

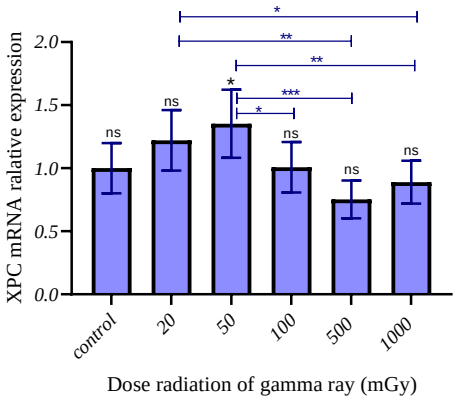

Figure 4. Effect of low-dose of gamma exposure on XPC gene expression in whole-body rat's peripheral lymphocytes 24 hours after irradiation. (Error bars indicated SD, *P<0.05, **P<0.01, ***P<0.001, and ns= Non-significant)

Compression of the gene expression alterations in response to low-dose (20 and 50 mGy) and higher ones (100, 500, and 1000 mGy):

As is evident from data shown in figure5, DDB2, GADD45A, and XPC expression remained unchanged in a dose of 20 mGy. It also appears that at doses above 20 mGy, they express differently. While XPC and GADD45A altered significantly at 50 mGy, DDB2 changed significantly after exposure to 100, 500, and 1000 mGy. Moreover, the extent of gene expression alteration at higher doses of 100, 500, and 1000 mGy seems more severe than that of their lower counterparts (50 mGy).

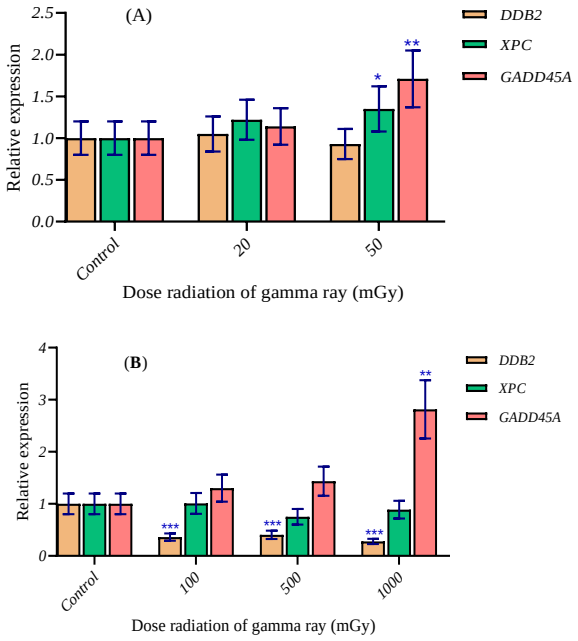

Figure 5. Gene expression analysis in rat peripheral blood lymphocytes after exposure to (A) low-dose (20, and 50 mGy) of gamma ray, and (B) the higher doses of gamma ray (100, 500, and 1000 mGy). (Error bars indicated SD, *P<0.05, **P<0.01, and ***P<0.001)

Discussion

The results of the present study indicated that the expression of DNA damage binding protein 2 (DDB2) due to whole-body irradiation down-regulated significantly ($P<0.001$) at doses of 100, 500, and 1000 mGy of gamma radiation. Data analysis also indicated that the expression of DDB2 has no significant change at doses less than 100 mGy. These results suggest a dose-dependent feature of DDB2 expression after low-dose radiation exposure.

Drug resistance is one adverse effect of a decline of DDB2 in cancer cells. In this regard, Stoyanova et al. [26] demonstrated the lack of P21 and consequent accumulation of P21 in DDB2-deficient cells as one reason for the inhibition of DNA-damaged apoptosis and drug resistance. Furthermore, based on research of the Oncomine database, as a P53-induced gene, DDB2 is one of the top 10% under-expressed genes in various cancer types, including brain and CNS, colon, breast, follicular lymphoma, leukemia, liver, ovarian, head, and neck SCC, lung and prostate [27]. Recently, Visweswaran et al. [28] showed that 67% of patients exposed to low-dose radiation during neuro-interventional radiology procedures represented a decreased expression of DDB2. The central role of DDB2 in modulating the homologous recombination pathway in patients who suffered from triple-negative breast cancer (TNBC) was reported by Zhao et al. [29]. Since TNBC is characterized by a high rate of recurrence and poor prognosis, they suggest assessing DDB2 expression would be a promising therapeutic target for TNBC [29]. The critical role of DDB2 in DNA-repair mechanisms is inevitable due to its preventative function against genome instability and cancer formation. In addition, it can also play a pivotal role in suppressing metastasis via inhibition of EMT-induced activators such as snail, zeb1, and VEGF [30]. Therefore, the downregulation of DDB2 can facilitate tumorigenesis, cancer metastasis, and therapy resistance. XPC is one of the three genes cooperating with DDB2 in the DNA damage site's cognition to inhibit nucleotide excision repair. Examining this gene expression in the current study indicated that XPC overexpressed significantly at a low dose of 50 mGy. However, at doses above 50 mGy, its expression remained unchanged compared to the control group. Evaluating the expression level of XPC, Li et al. [31] found that the expression level of XPC represents a time-dependent manner. They observed the up-regulation of XPC after 6 hours in post-irradiated blood samples from 30 healthy humans at doses of 0.5 and 1 Gy, while XPC expression reached a plateau 24 hours after radiation and then down-regulated at 48 h post-irradiation. Another study by Long et al. [32], verified that irradiation-induced transcription of the XPC gene is dose- and time-dependent.. They demonstrated that the 0.05 Gy low dose of a cobalt-60 γ-ray was effective in induction XPC expression in human lymphoblastoid AHH-1 cells with a maximum increase of about 1.93-fold 10 h after irradiation. However, in this study, we observed a fold change of about 1.35 in the expression level of XPC after the same exposure in rat blood lymphocyte samples 24 h after irradiation. This discrepancy may occur due to the differences in expression levels of XPC in human cells with that of the rodent cells and also the post-irradiated time. By evaluating the expression levels of GADD45A, a third P53-independent gene involved in NER, we found that radiation resulted in the upregulation of GADD45A at all given doses of gamma radiation; however, it was statistically significant just at doses of 50 and 1000 mGy ($P<0.01$). According to the data obtained from multiple comparison analyses, there was a significant change between various groups. Growth arrest and DNA damage gene 45, GADD45A, often induced by DNA damage and other environmental stress, signals which are involved in growth arrest and apoptosis [28, 33, 34]. GADD45A gene responds to stress signal normally through the activation of the p38/JNK pathway; however, it is reported that the GADD45 gene represents a complex response after exposure to ionizing radiation [35]. Amundson et al. [36] reported the induction of stress genes like CDKN1A and GADD45 in post-irradiated Ml-1 cells after exposure to low-doses of gamma-ray (2-50 cGy). They also reported a linear dose-response relationship for induction of GADD45 in these cells. Besides, Grace et al. [37] observed a linear upregulation in the GADD45 expression in whole blood samples from healthy human donors while their samples were exposed to 1, 2, and 3 Gy of gamma radiation. They suggested these changes in GADD45 as a suitable biomarker for radiation biodosimetry. Based on evidence reported by Wilson et al. [38], low dose irradiation (<1 Gy) of hESCs, resulted in GADD45A overexpression, which is then may facilitate activation of the P38/C-Jun NH2-terminal kinase pathway through MTK1/MEKK4 kinase and CXCL10, a chemokine for receptor CXCR3 that is involved in the recruitment of inflammatory cells. Recently Zhao et al. [39] used biochemically-inspired genomic machine learning as a promising method for biodosimetry testing and predicted DDB2 and GADD45A as the best human signatures with accuracies of up to 98%. Even though we observed some alteration in expression of DDB2, XPC, and GADD45A in rat lymphocyte sample within a 20-1000 mGy dose range of gamma exposure, no clear induction was observed for a linear dose-response distribution. Add to this, dividing the range of applied dose into low-doses of 20 and 50 mGy and the higher levels (100,500 and 1000 mGy) predicts that some genes, XPC, for instance, respond to low-dose radiation but not to higher adjacent dose radiation. In contrast, some like DDB2, acted reversely and remained unchanged at a lower dose. The existence of these differences between gene expression profiles may be evidence of the non-linear correlation. Interestingly, our data suggest that even various low-dose radiation may pose features that distinguish them from their near counterparts, which we notably observed in the GADD45A expression after irradiation. This varied behavior of low-dose may be explainable through

specific responses to low-dose, such as the adaptive response, the bystander effect, and the signaling pathways involved.

Conclusion

Immediate reaction to radiation-induced DNA damage is the activation of DNA repair mechanisms. Monitoring the expression of DNA-repair-related genes might be an advantageous way for identifying bio-dosimeters of radiation exposure, particularly for low-dose radiation. It was found in this study how different dosages of gamma radiation affected the expression of genes such DDB2, XPC, and GADD45A, which are involved in repairing DNA damage. Three essential genes involved in repairing DNA damage—DDB2, XPC, and GADD45A—have their expression considerably impacted by exposure to low levels of gamma radiation (less than 1 Gy). However, in order to identify whether or not these genes can serve as an effective biomarker for monitoring chronic low-level exposure in human, further studies have to be done on the changed expression patterns of other DNA-damaged repair genes following low-dose irradiation.

Acknowledgment

The present article was extracted from the thesis written by Manouchehr Ghasemi and financially supported by Shiraz University of Medical Sciences, Shiraz, Iran Grants No.90036. The authors would like to thank the Center for Development of Clinical Research of the Nemazee Hospital for statistical analysis assistance and editing the manuscript.

References

1. Håkansson P, Hofer A, Thelander L. Regulation of mammalian ribonucleotide reduction and dNTP pools after DNA damage and in resting cells. Journal of Biological Chemistry. 2006 Mar 24;281(12):7834-41.
2. Khanna A. DNA Damage in Cancer Therapeutics: A Boon or a Curse? Targeting DNA Damage in Cancer. Cancer research. 2015 Jun 1;75(11):2133-8.
3. Riley PA. Free radicals in biology: oxidative stress and the effects of ionizing radiation. International journal of radiation biology. 1994 Jan 1;65(1):27-33.
4. Yamamori T, Yasui H, Yamazumi M, Wada Y, Nakamura Y, Nakamura H, et al. Ionizing radiation induces mitochondrial reactive oxygen species production accompanied by upregulation of mitochondrial electron transport chain function and mitochondrial content under control of the cell cycle checkpoint. Free Radical Biology and Medicine. 2012 Jul 15;53(2):260-70.
5. Kurz EU, Lees-Miller SP. DNA damage-induced activation of ATM and ATM-dependent signaling pathways. DNA repair. 2004 Aug 1;3(8-9):889-900.
6. Valerie K, Yacoub A, Hagan MP, Curiel DT, Fisher PB, Grant S, Dent P. Radiation-induced cell signaling: inside-out and outside-in. Molecular cancer therapeutics. 2007 Mar;6(3):789-801.
7. Houtgraaf JH, Versmissen J, van der Giessen WJ. A concise review of DNA damage checkpoints and repair in mammalian cells. Cardiovascular Revascularization Medicine. 2006 Jul 1;7(3):165-72.
8. Torgovnick A, Schumacher B. DNA repair mechanisms in cancer development and therapy. Frontiers in genetics. 2015 Apr 23;6:157.
9. Bahreyni-Toossi MT, Fardid R, Rezaee A, Sadr-nabavi A, Rafatpanah H, Bolbolian M. Expression of apoptotic genes can distinguish radiation workers from normal population. International Journal of Low Radiation. 2012;8(5-6):388-99.
10. Hoglund, E. Blomquist, J. Carlsson, B. Stenerlow E. DNA damage induced by radiation of different linear energy transfer: initial fragmentation. International journal of radiation biology. 2000 Jan 1;76(4):539-47.
11. Penninckx S, Cekanaviciute E, Degorre C, Guiet E, Viger L, Lucas S, et al. Dose, LET and strain dependence of radiation-induced 53BP1 foci in 15 mouse strains ex vivo introducing novel DNA damage metrics. Radiation research. 2019 Jul;192(1):1-2.
12. Tu W, Dong C, Fu J, Pan Y, Kobayashi A, Furusawa Y, et al. Both irradiated and bystander effects link with DNA repair capacity and the linear energy transfer. Life sciences. 2019 Apr 1;222:228-34.
13. Rühm W, Azizova T, Bouffler S, Cullings HM, Grosche B, Little MP, et al. Typical doses and dose rates in studies pertinent to radiation risk inference at low doses and low dose rates. Journal of radiation research. 2018 Apr 1;59(suppl_2):ii1-0.
14. Desouky O, Ding N, Zhou G. Targeted and non-targeted effects of ionizing radiation. Journal of Radiation Research and Applied Sciences. 2015 Apr 1;8(2):247-54.
15. Little JB. Genomic instability and bystander effects: a historical perspective. Oncogene. 2003 Oct;22(45):6978-87.
16. Morgan WF. Is there a common mechanism underlying genomic instability, bystander effects and other nontargeted effects of exposure to ionizing radiation?. Oncogene. 2003 Oct;22(45):7094-9.
17. Hoeijmakers JH. DNA damage, aging, and cancer. New England Journal of Medicine. 2009 Oct 8;361(15):1475-85.
18. Hustedt N, Durocher D. The control of DNA repair by the cell cycle. Nature cell biology. 2017 Jan;19(1):1-9.
19. Bernstein C, Bernstein H, Payne CM, Garewal H. DNA repair/pro-apoptotic dual-role proteins in five major DNA repair pathways: fail-safe protection against carcinogenesis. Mutation Research/Reviews in Mutation Research. 2002 Jun 1;511(2):145-78.
20. Budworth H, Snijders AM, Marchetti F, Mannion B, Bhatnagar S, Kwoh E, et al. DNA repair and cell cycle biomarkers of radiation exposure and inflammation stress in human blood. PloS one. 2012 Nov 7;7(11):e48619.
21. Criswell T, Leskov K, Miyamoto S, Luo G, Boothman DA. Transcription factors activated in mammalian cells after clinically relevant doses of ionizing radiation. Oncogene. 2003 Sep;22(37):5813-27.
22. Fei P, El-Deiry WS. P53 and radiation responses. Oncogene. 2003 Sep;22(37):5774-83.
23. Gudkov AV, Komarova EA. The role of p53 in determining sensitivity to radiotherapy. Nature Reviews Cancer. 2003 Feb;3(2):117-29.

24. Puar YR, Shanmugam MK, Fan L, Arfuso F, Sethi G, Tergaonkar V. Evidence for the involvement of the master transcription factor NF-κB in cancer initiation and progression. Biomedicines. 2018 Jul 27;6(3):82.
25. Rao X, Huang X, Zhou Z, Lin X. An improvement of the 2ˆ (−delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. Biostatistics, bioinformatics and biomathematics. 2013 Aug;3(3):71.
26. Stoyanova T, Roy N, Kopanja D, Bagchi S, Raychaudhuri P. DDB2 decides cell fate following DNA damage. Proceedings of the National Academy of Sciences. 2009 Jun 30;106(26):10690-5.
27. Bagchi S, Raychaudhuri P. Damaged-DNA binding protein-2 drives apoptosis following DNA damage. Cell division. 2010 Dec;5(1):1-5.
28. Visweswaran S, Joseph S, Hegde V, Annalakshmi O, Jose MT, Perumal V. DNA damage and gene expression changes in patients exposed to low-dose X-radiation during neuro-interventional radiology procedures. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2019 Aug 1;844:54-61.
29. Zhao L, Si CS, Yu Y, Lu JW, Zhuang Y. Depletion of DNA damage binding protein 2 sensitizes triple-negative breast cancer cells to poly ADP-ribose polymerase inhibition by destabilizing Rad51. Cancer science. 2019 Nov;110(11):3543-52.
30. Wang YC, Huang JL, Lee KW, Lu HH, Lin YJ, Chen LF, et al. Downregulation of the DNA repair gene DDB2 by arecoline is through p53′s DNA-binding domain and is correlated with poor outcome of head and neck cancer patients with betel quid consumption. Cancers. 2020 Jul 25;12(8):2053..
31. Li S, Lu X, Feng JB, Tian M, Wang J, Chen H, et al. Developing gender-specific gene expression biodosimetry using a panel of radiation-responsive genes for determining radiation dose in human peripheral blood. Radiation Research. 2019 Oct;192(4):399-409.
32. Long XH, Zhao ZQ, He XP, Wang HP, Xu QZ, An J, et al. Dose-dependent expression changes of early response genes to ionizing radiation in human lymphoblastoid cells. International journal of molecular medicine. 2007 Apr 1;19(4):607-15.
33. Han N, Yuan F, Xian P, Liu N, Liu J, Zhang H, et al. GADD45a mediated cell cycle inhibition is regulated by P53 in bladder cancer. OncoTargets and therapy. 2019;12:7591.
34. Visweswaran S, Joseph S, Dhanasekaran J, Paneerselvam S, Annalakshmi O, Jose MT, et al. Exposure of patients to low doses of X-radiation during neuro-interventional imaging and procedures: Dose estimation and analysis of γ-H2AX foci and gene expression in blood lymphocytes. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2020 Aug 1;856:503237.
35. Li D, Dai C, Yang X, Li B, Xiao X, Tang S. GADD45a regulates olaquindox-induced DNA damage and S-phase arrest in human hepatoma G2 cells via JNK/p38 pathways. Molecules. 2017 Jan 13;22(1):124.
36. Amundson SA, Do KT, Fornace Jr AJ. Induction of stress genes by low doses of gamma rays. Radiation research. 1999 Sep 1;152(3):225-31.
37. Grace M, McLeland CB, Blakely WF. Real-time quantitative RT-PCR assay of GADD45 gene expression changes as a biomarker for radiation biodosimetry. International Journal of Radiation Biology. 2002 Jan 1;78(11):1011-21.
38. Wilson KD, Sun N, Huang M, Zhang WY, Lee AS, Li Z, et al. Effects of ionizing radiation on self-renewal and pluripotency of human embryonic stem cells. Cancer research. 2010 Jul 1;70(13):5539-48.
39. Zhao JZ, Mucaki EJ, Rogan PK. Predicting ionizing radiation exposure using biochemically-inspired genomic machine learning. F1000Research. 2018;7.