Assessment of Adaptive Response of Gamma Radiation in the Operating Room Personnel Exposed to Anesthetic Gases by Measuring the Relative Gene Expression Changes Ku80, Ligase1 and P53

Rajabi Pour M.1,2, Fardid R.3,4,*, Zare T.5, Kargar Shouroki F.6, Mosleh-Shirazi M. A.3,4, Behzad Behbahani A.7

ABSTRACT

Background: Some operating room personnel are occupationally exposed to genotoxic agents such as anesthetic gases and ionizing radiation. Adaptive response, as a defense mechanism, will occur when cells become exposed to a low dose of factors harming DNA (priming dose), which in the subsequent exposure to higher dose of those factors (challenging dose), show more resistance and sensibility.

Objective: The aim of this study was to investigate adaptive response or synergy of ionizing radiation in the operating room personnel exposed to anesthetic gases by evaluation of the relative gene expression changes of effective genes for DNA repair such as Ku80, Ligase1 and P53.

Material and Methods: In this case-control study, 20 operating room personnel and 20 nurses (who were not present in the operating room) as controls were studied. Venous blood samples were drawn from participants. In order to evaluate the adaptive response, a challenging dose of 2Gy gamma radiation was applied to blood samples. Moreover, RNA extraction and cDNA synthesis were performed. Gene expression level was studied by RT-qPCR and compared with the control group.

Results: Ligase1 and P53 expression in the operating room personnel was significantly higher than that of the control group before irradiation (P<0.001). Statistically, there was no significant difference in the Ku80 and P53 expression in the operating room personnel before and after irradiation.

Conclusion: Given the findings of this study, exposure to challenging dose of gamma radiation can induce adaptive response in expression of Ku80 and P53 genes in operating room personnel.

Keywords
Adaptive Response; Ionizing Radiation; Anesthetic Gases; Operating Room Personnel; Occupational Exposure; DNA Repair; Gene Expression; RT-qPCR

Introduction
Some operating room personnel (for example, in the orthopedic, urology, and cardiac angiography operating rooms) are occupationally exposed to genotoxic agents such as anesthetic gases and ionizing radiation [1-3]. Although ventilation and scavenging systems
that mainly reduce the concentration of anesthetic gases are widely used, the complete removal of these gases is next to impossible [3]. Studies have shown that long-term exposure to anesthetic gases can lead to increased oxidative stress, DNA damage, genotoxicity and carcinogenesis [3,4]. Additionally, operating room personnel are exposed to ionizing radiation during cardiothoracic, neuro-spine, orology and orthopedic surgeries. The number of these procedures has increased significantly in the last decade [4]. Occupational exposure of individuals to ionizing radiation in the operating rooms is a serious concern for the safety of personnel and patients. The adverse biological effects of ionizing radiation vary based on the duration of exposure, which generally increases the risk of cancer [5]. Invasive cardiologists are the most exposed to ionizing radiation among health professionals and subsequently lead to an increase in the rate of their somatic DNA damage [6]. Adaptive response, as a defense mechanism in living organisms, occurs when cells are exposed to low dose of a physical or chemical genotoxic agent (priming dose), which in the subsequent exposure to higher dose of the same or another genotoxic agent (challenging dose), show more resistance and less sensitivity; hence, the level of cell damage will be reduced [7]. The low levels of cell damage can trigger signaling pathways that activate the mechanisms of DNA repair. These changes reduce the level of damage caused by high doses of genotoxic agents such as ionizing radiation [8]. Adaptive response has been demonstrated in a variety of in vitro and in vivo systems with end points such as cell survival ratio, gene expression variations, chromosomal aberrations, DNA single and double-strand breaks, carcinogenesis, enzymatic and antioxidant changes, micronucleus induction, and biochemical tests [8-10]. The factors proposed to describe the mechanisms of induction of adaptive response include activating DNA repair mechanisms, inducing the synthesis of new proteins, producing antioxidant compounds, effective detoxification of free radicals, enhancing the immune system, and inducing apoptosis [8]. However, this phenomenon is variable. Sometimes, the damage will be reduced after the challenging dose, or it will have a synergistic or additive effects [11]. Adaptive response variations depend on the type of genotoxic agents, dose and dose rates, cell line, experimental design conditions, time interval between priming and challenging doses, cell cycle stage, P53 status, physiological status and genetic structure of the blood donor [12-13]. One of the biomarkers of adaptive response is gene expression changes; hence, the aim of our study was to investigate adaptive or synergistic effect of ionizing radiation in the operating room personnel exposed to anesthetic gases by evaluating the relative expression changes of effective genes in DNA repair, such as Ku80, DNA Ligase1(Lig1) and P53. Moreover, determining a suitable biomarker for adaptive response was studied. These genes were selected based on recent adaptive response studies [11,14,15]. In this study, we considered chronic doses of anesthetic gases as priming dose, that operating room personnel are exposed as occupational exposure during their professional work, and in order to evaluate the adaptive response, a challenging dose of 2Gy gamma radiation for groups was used.

### Material and Methods

#### Study population and sampling

In this case-control study, the exposed group consisted of 20 personnel working in Shiraz Shahid Beheshti Hospital’s operating room (physician, nurse, technician), including 12 men and 8 women aged between 27 and 51 years with the mean age of 34.35± 7.33 years. These individuals had a history of exposure to anesthetic gases, such as N2O, Isoflurane, and Sevoflurane for at least 3 years and worked in the operating room for at least 6-h per day. The control group consisted of 20 nurses work-
Adaptive Effects and Anesthesia Gas Inhalation

ing in other wards of the hospital, including 12 men and 8 women aged between 25 and 48 years with the mean age of 34.05 ± 6.50 years, who had no occupational exposure to anesthetic gases and ionizing radiation. Demographic data, work experience, alcohol consumption, smoking, medical and genetic history, and history of exposure to chemicals and ionizing radiation were collected through standard questionnaires. The test and control groups were matched for age, gender, lifestyle and smoking habits. If the personnel were recently exposed to ionizing radiation and had any previous or current exposure to other chemical pollutants with genotoxic effects, chronic diseases, alcohol consumption, and smoking habits, they were excluded from the study. About 5 ml of the blood sample was obtained from each donor in EDTA containing vials after obtaining their written informed consent, which was approved by the local Ethics Committee of Shiraz University of Medical Sciences. Each whole blood sample was divided into two equal parts, one was kept as control and the second was exposed to challenging dose of 2Gy gamma radiation.

Irradiation

For adaptive response experiment, blood samples were irradiated at room temperature with a dose of 2Gy gamma radiation as a challenging dose at dose rate of 70 cGy/min (SSD: 50cm) using 60Co gamma-ray source in radiotherapy department of Namazi Hospital, Shiraz, Iran.

RNA Extraction and cDNA Synthesis

Thirty minutes after irradiation, from the irradiated and non-irradiated blood samples total RNA was extracted by the RNX-PLUS Kit (Sina Clon, Iran) according to the kit protocols and quantified using spectrophotometer (HELMA, USA). RNA integrity was confirmed by a 2% agarose gel electrophoresis. cDNA synthesis was done, using RevertAid first Strand cDNA synthesis kit (Takara, Japan) based on the manufacture’s protocol.

Real-Time Quantitative polymerase chain reaction (RT-qPCR)

The RT-qPCR reaction was designed after determining the concentration of cDNA, using the designed primers. Primers were designed using the Allele ID7 software (Premier Biosoft International, Palo Alto, USA). To eliminate genomic DNA contamination, the primer design was performed in the exon regions and synthesized by Bioneer Corporation (South Korea), which is shown in Table 1. The β-actin gene was used as the endogenous reference. The mRNA expression was quantitated for Lig1, Ku80, and P53 genes through

| Primer Name | Sequence (5’-3’) | Product size (bp) |
|-------------|------------------|-------------------|
| Ku80 PR-1(forward) | CGACAGGTGGTTTGCCTAGAA | 223 |
| Ku80 PR-2 (reverse) | TCACATCCATGCTCACGATT | |
| P53 PR-1(forward) | TGGCCATCTACAAGCAGTCA | 212 |
| P53 PR-2 (reverse) | GGTCAGTCAGAGCCAACCT | |
| Lig1 PR-1(forward) | AGATCCAGCCATTCAAAGTG | 194 |
| Lig1 PR-2 (reverse) | GAAGACAAACTCGCCCTTGG | |
| β– actin PR-1 (forward) | GGGAAATCGTGCGTGACATTAAGG | 183 |
| β– actin PR-2 (reverse) | GGAAGGAAGGCTGGAAGAAGTG | |

Table 1: Primer sequences used for RT-qPCR
real-time quantitative polymerase chain reaction (RT-qPCR) (Applied Biosystems™, ABI, USA). Each RT-qPCR reaction was performed in a total volume of 20μL containing 2x SYBR Green qPCR Master Mix (Yekta Tajhiz Azma, Iran) (10μL), primers (2μL-10μmol), deionized water (6μL), and cDNA (2μL). The cycling conditions were as follows: 2 minutes at 95°C (Activation) and 45 cycles (15 s at 95°C (Denaturation), 1 minute at 62°C (Annealing), 1 minute at 72°C (Extension), and 15 s At 95°C (Final Extension). In order to optimize the reaction, using different dilutions of the PCR product, standard charts and reaction efficiency for Lig1 (82%), Ku80 (98%), P53 (95%) and β-actin (82%) were obtained. The results were analyzed using \((R = 2^{-ΔΔC_t})\) and the reference gene β-actin for normalization. Thus, for the completely studied population, the relative expression of Lig1, Ku80, and P53 was obtained. Table 1 shows primer sequences used for RT-qPCR.

Verification polymerase chain reaction and primer design

To verify Lig1, Ku80, P53 and β-actin primers with 194, 223, 212 and 183 base pair (bp) band lengths, PCR was performed with a cDNA sample. 5μL of PCR product was loaded on 2% agarose gel. The distinctly mentioned band lengths of each gene were visible after DNA Safe Staining (Sina Clon, Iran) (Figure 1).

Statistical analysis

Statistical analysis was performed using SPSS software version 21. The level of significance was set at \(P < 0.05\). Data are represented as mean ± SD. The paired t-test was used to compare the mean of gene expression changes in each group before and after irradiation. Regarding the normal distribution of data by the Kolmogorov–Smirnov test, one-way ANOVA with post-hoc Tukey Test was used to compare the mean of gene expression amongst groups.

*Figure 1:* Electrophoresis image of the designed Lig1, P53, Ku80 and β-actin primers (194, 212, 223 and 183 bp) which visible in the image.
Adaptive Effects and Anesthesia Gas Inhalation

(operating room personnel and control group in before and after irradiation).

Results

In this study, adaptive response study was performed using gene expression changes in 40 individuals (operating room personnel and control group) after 30 min of challenging dose administration.

The demographic characteristics of the studied subjects are presented in Table 2. There were no significant differences between the groups as far as demographic variables were concerned. None of the participants in both groups was smokers.

In the present study, the control group and operating room personnel were divided into two groups before and after irradiation, based on the challenging dose of ionizing radiation and mean gene expression values for groups are shown in Figures 2, 3 and 4. (Control, Control (+IR), ORP, ORP (+IR)).

The expression of Lig1 in the operating room personnel had a significant increase in

### Table 2: Data for control group and Operating room personnel obtained from the questionnaire

| Groups                      | Sample size | Age (years) Mean ± SD | Gender (M or F) | Employ in year Mean ± SD | Smoking | Radiation or anesthetic gases exposure |
|-----------------------------|-------------|------------------------|-----------------|--------------------------|---------|---------------------------------------|
| Control                     | 20          | 34.05 ± 6.50           | 12(M) 8(F)      | 10.39± 6.31              | No      | No                                    |
| Operating room personnel    | 20          | 34.35± 7.33            | 12(M) 8(F)      | 9.45± 6.85               | No      | Anesthetic gases                      |

**Figure 2:** Comparing the relative expression of Lig1 in the control group vs. operating room personnel (ORP) in before and after irradiation. IR: ionizing radiation (2Gy) (**P<0.001).
comparison with the before irradiation control group (P<0.001) (Figure 2). However, no significant decrease in the expression of Lig1 was seen after irradiation in the control group (P>0.05). In contrast, Lig1 expression significantly decreased in the operating room personnel after irradiation (P<0.001).

The expression of Ku80 in the control group after irradiation was significantly higher than before radiation (P<0.05) (Figure 3). However, there was no significant difference in the Ku80 expression in the operating room personnel in before and after irradiation (P>0.05).

The expression P53 in the control group after irradiation showed a significant difference compared to the before irradiation (P<0.001) (Figure 4). Moreover, the comparison of P53 expression in the operating room personnel showed a significant difference compared to the control group in before irradiation (P<0.001). However, there was no significant change in the expression of P53 in the operating room personnel in before and after irradiation (P>0.05).

**Discussion**

The present study aims to assess the adaptive response of gamma radiation in the operating room personnel exposed to anesthetic gases by measuring the relative gene expression changes Ku80, Ligase1 and P53.

A recent study in the target hospital of our study, the mean concentration of anesthetic gases is reported exceeding the global standard (NIOSH) in operating room staff. Moreover, the micronucleus (MN) induction and chromosomal abnormalities in these staff are reported with significant increase compared to the control group. This matter can be a result of extensive application of anesthetic gases, undesirable ventilation and scavenging systems, leakage of anesthetic devices and patients’ mask, and high flow rate of anesthetic gases in the operating room [16].

---

**Figure 3:** Comparing the relative expression of Ku80 in the control group vs. operating room personnel in before and after irradiation (*P<0.05).
In the present study, Lig1 expression in the operating room personnel in before irradiation had significantly increased compared to the control group. This could be explained by the fact that due to exposure to anesthetic gases and the genetic damages caused by it, the Lig1 plays an active role in repairing the damaged DNA, thereby it has a higher expression. After irradiation, Lig1 expression had significantly decreased in the operating room personnel and showed a high sensitivity to ionizing radiation. Lig1 has an important role in nucleotide excision repair (NER) pathway and the long base excision repair pathway (BER). Therefore, decrease in Lig1 activity contributes to genome instability and carcinogenesis [17]. In accordance with the present results, previous studies have shown the upregulation of Lig1 in human primary fibroblasts was observed within 24h after exposure to UV-C radiation [18].

In the present study, the expression of Ku80 in the control group following irradiation significantly increased. Some studies have shown the upregulation of Ku80 expression in human lymphocytes after exposure to priming dose of 0.1Gy and challenging dose of 2Gy after 4h [15]. DSBs is the most important DNA damage that seriously threatens the protection of genetic and epigenetic information. DSBs in human cells is restored through two important pathways namely homologous recombination (HR) and the path of non-homologous end joining (NHEJ). In the G0/G1 phase of the cell cycle, NHEJ is preferred as the principal route in restoring DSBs in human cells. The Ku Heterodimer, as a large protein required for the NHEJ pathway in mammalian cells, consists of Ku70 and Ku80 [15]. DSBs repair is reported as the key mechanistic approach in adaptive response [19].

**Figure 4**: Comparing the relative expression of P53 in control group vs. operating room personnel in before and after irradiation (***P<0.001).
In the present study, Ku80 and P53 expression in the operating room personnel did not change significantly before and after irradiation. It seems that exposure to anesthetic gases has caused adaptive effects in this group; hence, no significant changes were observed following 2Gy of gamma radiation. Additionally, upregulation of P53 in operating room personnel in comparison with before irradiation control group, could be explained by the fact that due to the presence of DNA damage in the operating room personnel exposed to anesthetic gases, P53 expression increased and suggesting the active role of this gene in repairing these damages. P53 regulates the cell cycle, DNA repair and apoptosis [20]. A significant increase in P53 expression level was observed at priming dose of 0.1, 0.3 and 0.6 followed by 2Gy of challenging dose at 4h and reported an adaptive response at 1 and 5h after the challenging dose [14]. Similarly, in another study, P53 expression in radiation workers was significantly higher than that of the control group and showed that low and chronic doses of ionizing radiation play an important role in increasing the expression of P53 amongst radiation workers [21]. In several studies, the role of P53 in radio-adaptive response was reported [22-24]. Regarding the results of our research and previous studies, P53 expression is important for the safety of workers exposed to genotoxic agents such as ionizing radiation and anesthetic gases. In our study, it seems that P53 expression in the operating room personnel reflects the constant presence of anesthetic gases in the workplace, and the resulting stress to the cell leads to an increased level of P53 for monitoring DNA damage.

The present study may have some limitations. For instance, it is the first that has investigated the radio-adaptive response using gene expression changes in the operating room personnel and there is no similar study in this regard. Therefore, for more investigation of the adaptive response in the operating room personnel, further studies are recommended by evaluating changes in the expression of other genes, different challenging doses, and a different time intervals for examining the gene expression after the challenging dose of ionizing radiation and investigation of other biomarkers such as enzymatic and antioxidant changes and other techniques such as Comet and MN assay. For instance, a cellular adaptive response to chronic exposure to low-dose radiation in interventional cardiologists through significant increasing of three antioxidant and apoptosis factors, was reported following 2Gy in vitro irradiation [6]. Additionally, the radio-adaptive response for radiation workers was studied with a challenge dose of 4Gy of gamma rays by the use of neutral comet assay. Results of this study indicated a significant decrease in DNA damages for the radiation workers compared to the control subjects [19].

One of the limitations of this study is that the type of target or biomarker is of particular importance in examining the radio-adaptive response. In fact, there is a wide range of genes exhibiting different responses to ionizing radiation [25]. However, the genes evaluated in the present study were selected based on the results of recent studies in radio-adaptive response and confirmation of articles [11,14,15].

**Conclusion**

Given the findings of this study, exposure to the challenging dose of gamma radiation could induce an adaptive response in the expression of Ku80 and P53 genes in the operating room personnel. In addition, these genes can be considered as suitable biomarkers in radio-adaptive response studies. However, this does not mean to ignore the rules of radiation protection in the operating rooms. According to recommendations of the International Commission of Radiological Protection (ICRP), the operating room personnel required to use personnel dosimeters such as TLD dosimeter for monitoring of radiation dose and the received dose should not exceed 20 mSv/year.
Moreover, considering the high level of P53 expression and Lig1 in the operating room personnel in before irradiation, it is necessary to pay more attention and make more precise decisions regarding the health status and medical care of these people.

Acknowledgment
The present article was extracted from the thesis written by Mahdi Rajab Pour and was financially supported by Shiraz University of Medical Sciences grants No. 14074 The authors wish to thank Mr. H. Argasi at the Research Consultation Center (RCC) of Shiraz University of Medical Sciences for his invaluable assistance in editing this manuscript.

Conflict of Interest
None

References
1. Volquind D, Bagatini A, Monteiro GMC, Londoero JR, Benvenutti GD. Occupational hazards and diseases related to the practice of anesthesiology. *Braz J Anesthesiol.* 2013;63(2):227-32. doi: 10.1016/S0034-7094(13)70221-6. PubMed PMID: 23601267.

2. Maghsoudi B, Mortazavi SMJ, Khademi S, Vatanikhah S. Evaluation of Radiation Exposure Pattern and Radiation Absorbed Dose Resulting from Occupational Exposure of Anesthesiologists to Ionizing Radiation. *J Biomed Phys Eng.* 2017;7(3):271-8. PubMed PMID: 29082218.

3. Deng HB, Li FX, Cai YH, Xu SY. Waste anesthetic gas exposure and strategies for solution. *Journal of Anesthesia.* 2018;32:269-82. doi: 10.1007/s00540-018-2448-1. PubMed PMID: 29404778.

4. Abuzaid MM, Elshami W, Hasan H. Knowledge and Adherence to Radiation Protection among Healthcare Workers at Operation Theater. *Asian Journal of Scientific Research.* 2019;12(1):54-9. doi: 10.3933/ajsr.2019.54.59.

5. Mohsfegh S, Hasanzadeh H, Jadidi M, Mirmohammadkhani M, Bizarafan-Rajabi A, et al. Evaluation of knowledge, attitude and practice of personnel in operating room, ERCP, and ESWL towards radiation hazards and protection. *Middle East J Rehabil Health Stud.* 2017;4(3):e12354. doi: 10.5812/mejrh.12354.

6. Russo GL, Tedesco I, Russo M, Cioppa A, Andreassi MG, Picano E. Cellular adaptive response to chronic radiation exposure in interventional cardiologists. *European Heart Journal.* 2012;33(3):292-5. doi: 10.1093/eurheartj/ehr288.

7. Ramachandran EN, Karuppasamy CV, Kumar VA, Soren DC, Kumar PR, Koya PKM, Jaikrishnan G, Das B. Radio-adaptive response in peripheral blood lymphocytes of individuals residing in high-level natural radiation areas of Kerala in the southwest coast of India. *Mutagenesis.* 2017;32(2):267-73. doi: 10.1093/mutage/gew057.

8. Dimova EG, Bryant PE, Chankova SG. Adaptive response - Some underlying mechanism and open questions. *Genetics and Molecular Biology.* 2008;31(2):396-408. doi: 10.1590/S1415-47572008000300002.

9. Nenoi M, Wang B, Vares G. In vivo radioadaptive response: a review of studies relevant to radiation-induced cancer risk. *Hum Exp Toxicol.* 2015;34(3):272-83. doi: 10.1177/0960327114537537. PubMed PMID: 24925363. PubMed PMCID: PMC4442823.

10. Wang B, Tanaka K, Ninomiya Y, Maruyama K, Varès G, et al. Increased Hematopoietic Stem Cells/Hematopoietic Progenitor Cells Measured as Endogenous Spleen Colonies in Radiation-Induced Adaptive Response in Mice (Yonezawa Effect). *Dose-Response.* 2018;16(3):1559325818790152. doi: 10.1177/1559325818790152. PubMed PMID:30150909. PubMed PMCID: PMC6104214.

11. Toprani SM, Das B. Radio-adaptive response of base excision repair genes and proteins in human peripheral blood mononuclear cells exposed to gamma radiation. *Mutagenesis.* 2015;30(5):663-76. doi: 10.1093/mutage/gev032.

12. Wolff S, Jostes R, Cross FT, Hui TE, Afzal V, Wiencke JK. Adaptive response of human lymphocytes for the repair of radon-induced chromosomal damage. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis.* 1991;250(1-2):299-306. doi: 10.1016/0027-5107(91)90185-Q.

13. Shibamoto Y, Nakamura H. Overview of Biological, Epidemiological, and Clinical Evidence of Radiation Hormesis. *International journal of molecular sciences.* 2018;19(8):2387. doi: 10.3390/ijms19082387.

14. Saini D, Shelke S, Vannam AM, Toprani S, Jain V, Das B, Seshadri M. Transcription profile of DNA damage response genes at G0 lymphocytes exposed to gamma radiation. *Molecular and Cellular Biochemistry.* 2012;364(1-2):271-81. doi: 10.1007/s11010-012-1227-9.
15. Shelke S, Das B. Dose response and adaptive response of non-homologous end joining repair genes and proteins in resting human peripheral blood mononuclear cells exposed to γ radiation. *Mutagenesis*. 2015;30(3):365-79. doi: 10.1093/mutage/geu081.

16. Shouroki FK, Neghab M, Mozdarani H, Alipour H, Yousefinejad S, Fardid R. Genotoxicity of inhalational anesthetics and its relationship with the polymorphisms of GSTT1, GSTM1, and GSTP1 genes. *Environmental Science and Pollution Research*. 2019;26(8):3530-41. doi: 10.1007/s11356-018-3859-0.

17. Li D, Li R, Zhang J, Li K, Wu Y. Association Between the LIG1 Polymorphisms and Lung Cancer Risk: A Meta-analysis of Case–Control Studies. *Cell Biochemistry and Biophysics*. 2015;73:381-7. doi: 10.1007/s12013-015-0619-3.

18. Montecucco A, Savini E, Biamonti G, Stefanini M, Focher F, Ciarrocchi G. Late induction of human DNA ligase I after UV-C irradiation. *Nucleic Acids Research*. 1995;23(6):962-6. doi: 10.1093/nar/23.6.962.

19. Pakniat F, Mozdarani H, Nasirian B, Faeghi F. Radioadaptive response in peripheral blood leukocytes of occupationally exposed medical staff with investigation of DNA damage by the use of neutral comet assay. *Int J Radiat Res*. 2013;11:91-7.

20. Adimoolam S, Ford JM. p53 and regulation of DNA damage recognition during nucleotide excision repair. *DNA repair*. 2003;2(9):947-54. doi: 10.1016/S1568-7864(03)00087-9.

21. Mohammadi S, Gharati MR, Alang KEK, Rajaei R. Comparison of P53 Expression between Occupationally Exposed to Ionizing Radiation and Control Group. *Razi Journal of Medical Sciences*. 2011;18(91):36-43.

22. Jain V, Das B. Global transcriptome profile reveals abundance of DNA damage response and repair genes in individuals from high level natural radiation areas of Kerala coast. *PLoS One*. 2017;12(11):e0187274. doi: 10.1371/journal.pone.0187274. PubMed PMID: 29161272. PubMed PMCID: PMC5697823.

23. Cheng GH, Wu N, Jiang DF, Zhao HG, Zhang Q, Wang J-F, Gong Sh-L. Increased levels of p53 and PARP-1 in EL-4 Cells Possibly Related with the Immune Adaptive Response Induced by Low Dose Ionizing Radiation in vitro. *Biomedical and Environmental Sciences*. 2010;23(6):487-95. doi: 10.1016/S0895-3988(11)60012-3.

24. Zhao Y, Zhong R, Sun L, Jia J, Ma S, Liu X. Ionizing Radiation-Induced Adaptive Response in Fibroblasts under Both Monolayer and 3-Dimensional Conditions. *PLoS One*. 2015;10(3):e0121289. doi: 10.1371/journal.pone.0121289. PubMed PMID:25807079. PubMed PMCID:PMC4373882.

25. Amundson SA, Lee RA, Koch-Paiz CA, Bittner ML, Meltzer P, Trent JM, Fornace AJ. Differential Responses of Stress Genes to Low Dose-Rate γ Irradiation1 DOE Grant ER62683. *Molecular Cancer Research*. 2003;1(6):445-52.