Radiation-induced Non-targeted Effect and Carcinogenesis; Implications in Clinical Radiotherapy

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ABSTRACT

Bystander or non-targeted effect is known to be an interesting phenomenon in radiobiology. The genetic consequences of bystander effect on non-irradiated cells have shown that this phenomenon can be considered as one of the most important factors involved in secondary cancer after exposure to ionizing radiation. Every year, millions of people around the world undergo radiotherapy in order to cure different types of cancers. The most crucial aim of radiotherapy is to improve treatment efficiency by reducing early and late effects of exposure to clinical doses of radiation. Secondary cancer induction resulted from exposure to high doses of radiation during treatment can reduce the effectiveness of this modality for cancer treatment. The perception of carcinogenesis risk of bystander effects and factors involved in this phenomenon might help reduce secondary cancer incidence years after radiotherapy. Different modalities such as radiation LET, dose and dose rate, fractionation, types of tissue, gender of patients, etc. may be involved in carcinogenesis risk of bystander effects. Therefore, selecting an appropriate treatment modality may improve cost-effectiveness of radiation therapy as well as the quality of life in survived patients. In this review, we first focus on the carcinogenesis evidence of non-targeted effects in radiotherapy and then review physical and biological factors that may influence the risk of secondary cancer induced by this phenomenon.

Keywords

Radiation, Bystander Effect, Carcinogenesis, Non-targeted Effect, Secondary Cancer, Genomic Instability

Introduction

Radiation therapy including external radiotherapy and brachytherapy is an indispensable part of cancer treatment modalities, with more than half of all cancer patients who have undergone radiotherapy. This includes millions of people around the world [1]. So, improvement in radiotherapy efficiency associated with reduction of early and late side effects of exposure to radiation are most important aims in this way. However, in recent years the increased life expectancy results in growing concerns related to long term consequences of radiotherapy including secondary malignancy. It may result in decline in the quality of life among patients who have undergone radiotherapy and also affect cost-effectiveness of radiotherapy, particularly pediatric patients [2, 3]. The perception of biological effects of ionizing radiation can help manage side effects of radiotherapy such as secondary cancers by selecting an appropriate radiation treatment modality.

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Received: 28 December 2016
Accepted: 18 Janurary 2017

Non-targeted or distant bystander effect is a phenomenon in radiotherapy which causes damage to non-irradiated cells in distant tissues. The bystander effect is being observed in different cell types with different end points, especially carcinogenesis markers. This phenomenon may lead to systemic effects in patients who have undergone local radiotherapy for a certain part of the body [4]. Moreover, it is reported that bystander effects may be linked to secondary cancers in patients who have undergone radiation treatment [5]. Carcinogenesis effects of bystander phenomenon in animal models have been confirmed. A study by Mancuso et al. demonstrates that partial irradiation can result in cancer induction in non-targeted tissues. They used a radiosensitive Patched-1 (Ptch1) heterozygous mice model to evaluate genetic damage and the induction of medulloblastoma in a non-targeted brain after the irradiation of mice with skull shield. Lower half of the body of mice was irradiated (3 Gy of X-rays) while upper organs were protected. The results showed increased medulloblastoma rate in non-targeted brains. Increased risk of carcinogenesis was associated with chromosome damage and apoptosis in non-targeted cerebellums [6]. Considering the pivotal role of bystander effects in risk of secondary carcinogenesis, efforts to understand the basic mechanisms and modulate the genetic damages induced by this phenomenon may provide new approaches to cancer management.

Evidence for the High Incidence of Secondary Cancers in Out-of-field Organs

Based on cellular and molecular effects investigated with the bystander effect, there are concerns related to the incidence of secondary cancers following radiotherapy. To select the most appropriate treatment method, the awareness of the probability of secondary malignancies after treatment should be heightened. The increased risk of secondary cancers reduces the weight of benefits of radiation therapy against the adverse effects. Probably, the best example for the involvement of the bystander effect in cancer induction is high incidence of lung cancer among patients who have had external radiotherapy and brachytherapy due to the treatment of pelvis cancers such as prostate, ovarian and rectal cancers. Induction of second cancers were obvious particularly for long term survivors [7].

In addition to in-field secondary cancers, Bostrom et al. declared an increase in secondary cancers in out-of-field area after radiotherapy for cancers such as lung, sarcomas and melanoma [8]. Moreover, Joung et al. contended the increased frequency of out-of-field cancers after radiotherapy for prostate cancer the same as esophagus, stomach, liver, pancreas, larynx, lung, bronchus and thyroid cancers. These results were obtained from follow-up of 55,378 men diagnosed with primary prostate cancer [9]. Brenner et al. compared secondary cancer induction in 51,584 men with prostate cancer who underwent radiotherapy and 70,539 men who underwent surgery without radiotherapy. They proclaim that the most prevalent secondary cancers among the irradiated group compared to the surgery group are bladder, rectum and lung cancers [10].

The increased risk of lung cancer is also reported after radiotherapy for cervical cancer, in a cohort comprising 86,193 patients. While, the reported average radiation dose received by lung tissue during radiotherapy for cervical cancer had 0.1 Gy, the second excessive lung cancer, which is anatomically distant from the irradiated organs including half of excess cancers; although, authors proposed possible roles of smoking for increased lung cancer in this cohort study [11]. The analysis of the frequency of secondary malignancies in patients who have undergone radiotherapy for rectal cancer showed a significant increase in secondary cancers in in-field-of-treatment organs, and also out-of-field organs such as lungs, the stomach and the colon. The radia-

tion doses received by such organs were very low compared to the doses received by in-field of treatment organs. The authors state that the explanation for increased cancers induction in out-of-field organs considering the received doses is not obvious [12]. It seems that risk of secondary cancer induced by scattered radiation in out-of-field organs is negligible, and increased cancer induction in out-of-field organs results in biological pathways [13].

Role of Irradiation Modalities on Bystander Responses

Nowadays, radiotherapy is used to treat many different cancers with high dose rates and heavy particles. Moreover, several new centers equipped with heavy ion accelerators are under way, But, cost-effectiveness and risk of secondary malignancies for these radiation modalities are open to debate. The bystander effect as a phenomenon involved in secondary cancer induction is likely to be the key factor in the selection of an appropriate modality for cancer treatment.

Radiation Quality

Although for the first time the non-targeted effects of ionizing radiation were investigated after local irradiation of protons, it quickly was demonstrated that this phenomenon could be induced by different radiation qualities. The perception of the effects of radiation quality on bystander cells is crucial to predict the radiation risks such as carcinogenesis associated with cancer radiotherapy by heavy ions and also space exploration [4].

The results of some studies on various qualities of radiation have confirmed that the bystander effect depends on the quality of radiation. The evaluation of bystander effects in human lymphocytes co-cultured with macrophages, irradiated with heavy carbon ions or γ-rays revealed more persistent upregulation of MAPKs in bystander cells [14]. Chronic oxidative damage and micronuclei formation were investigated for 20 population doublings of cells co-culture with cells that had been exposed to 56Fe and 600 MeV/u 28Si ions. However, it was not observed for lower LET radiation including 1 GeV proton ions. Chronic oxidative damage in progeny cells may be related to the function of respiratory chain complexes and the suppression of antioxidant enzymes including catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities [15]. In another study, the results of the comparison of micronucleus formation with X-ray, and carbon, neon and argon ions have shown that the number of micronuclei in bystander cells was greater for higher LET. Furthermore, it should be pointed that micronucleus formation occurred in a dose-dependent manner for all radiation qualities [16].

Anzenberg et al. evaluated the surviving fraction, γ-H2AX focus and micronuclei formation after irradiation with 250 kVp X-rays or α particles. The results exhibited greater γ-H2AX foci per nucleus and more obvious decrease in the surviving fraction after irradiation with X-ray compared to α particles [17]. Besides, it was investigated that oxidative damage and mutation in the progeny of bystander cells have a negative relationship to the quality of radiation. In a study, it was concluded that the next generation of bystander cells irradiated with X-rays and protons bears persistent oxidative stress, albeit it was not observed on cells irradiated by carbon ions [18].

Dose and Dose Rate Effects

In radiation therapy, irradiation with different dose rates such as brachytherapy, 60CO, LINAC, etc. is used to treat cancer. Each of these modalities contributes to different radiation doses received by normal tissues. So, use of each radiation treatment modality may lead to different damage levels in distant non-targeted tissues. The results are obtained from a study in which the bystander effect was investigated by different radiation doses and dose rates including external or radioactive seeds [19]. Liu et al. evaluated dose threshold for induction of the bystander effect in human

skin cell lines. Cells were irradiated in the range of 0.04 mGy-5 Gy, and then medium was transferred to non-irradiated cells. The results showed a dose threshold around 2 mGy for a change in cell survival and calcium flux. However, below that dose, survival fraction increased in bystander cells [20].

Gow et al. evaluated the effect of different dose rates of 60Co and 20 MeV electron irradiations in bystander responses to HPV-G cells. The dose rates included 1.1, 1.7, 3 and 4.7 Gy/min at different radiation doses; 0.5, 5 and 10Gy. The results did not show any significant differences in the survival fraction between different dose rates of 60Co. Besides, for 20 MeV electron, the effect of dose rate was investigated only for 10Gy, not for lower doses. Interestingly, cells that received medium from cells irradiated with 10Gy showed higher survival fractions. The authors proposed that it might be related to larger amounts of growth factors such as TGFβ that they stimulate cell proliferation, resulting in increase in survival fractions [21]. In very low and high doses, it seems that radiation bystander signals stimulate proliferation, but in other doses usually used in clinical applications, the bystander effect has a negative effect on cells. Therefore, such results indicate that the effect of radiation dose is deemed to be more substantial when compared to the dose rate.

Fractionation Effect

Mothersill et al. evaluated the effects of fractionation on bystander cells. They proved that the effect of fractionated irradiation was more obvious compared to the effect of a single dose. These results suggest that although the fractionated radiotherapy bears less toxicity to normal tissues which have received direct exposure, distant cells that received bystander signals from the irradiated tissue, are at a greater risk of carcinogenesis compared to single irradiation. This effect would attenuate sparing effects of the fractionated radiotherapy and may reduce the therapeutic ratio [22].

Another study did not show any differences in the frequency of micronucleus between a single acute dose and the fractionated one [23]. It is proposed that the effect of fractionation on the bystander effect response hinges on the radiation dose and seems to be obvious in higher doses [24]. In this case, in-vivo studies are very limited. Ilnytskyy et al. studied the effect of both acute and fractionation irradiation on the bystander effect in mice model study. They investigated that the skull irradiation with single or two-fractionated exposure results in similar DNA hypomethylation in the spleen. The results showed that dose-fractionation did not decrease DNA damage and long-term changes of DNA methylation compared to single irradiation [25]. As the bystander effect depends on the type of cell, the experimental design and the culture media, it is predictable that different studies yield various results. Moreover, most of these results are based on in-vitro studies and need to be approved by in-vivo experiments.

Sex Specificity of the Bystander Effect

Several years ago, studies demonstrated radiation-sensitivity is likely to be a phenomenon based on sex specificity [26]. A remarkable body of research has demonstrated the role of sex in DNA damage and subsequent consequences. Evidence has shown that patterns of gene expression, epigenetic changes and also secondary cancers induced by ionizing radiation occur on different frequencies in males and females [27-30]. For the first time, Korturbash et al. have shown that local cranial irradiation of mice results in a sex-dependent induction of DNA damage and alterations in global DNA methylation in spleen. It is confirmed that loss of DNA methylation is associated with genome rearrangements and increased risk of carcinogenesis. They investigated that sex hormones are responsible for the induction of different responses in non-targeted spleen tissue. The evaluation of the levels of global cytosine DNA methylation indicated

a significant and persistent hypomethylation in direct irradiated and the non-targeted spleen of male mice at 4 and 96 hours after exposure. In females, hypomethylation was less pronounced and seen only 96 hours after direct irradiation of whole body. The results showed sex specificity for proliferation and apoptosis induced by the irradiation of whole body and non-targeted effects [31].

Moreover, miRNA microarray analysis has revealed that the deregulation of microRNAome in the spleen tissue follows whole body or cranial irradiation. The results showed downregulation of 6 microRNAs in male mice, upregulation and downregulation of 3 and 6 microRNAs, respectively in females, thereby, confirming the role of sex hormones in the deregulation of microRNAome [32]. Accordingly, following studies confirmed that the induction of genes involved in redox system such as TGFβ and COX-2 in male mice are higher compared to that in females [33-34]. The upregulation of these genes results in the production of ROS and NO which leads to DNA damage and mutagenesis. However, risk of carcinogenesis after exposure to ionizing radiation based on sex is contradictory. Some studies have shown higher risk for females, while some have mentioned higher radiation sensitivity for male mice [35-37].

Tissue Specificity of the Bystander Effect

Different tissues have shown various responses to ionizing radiation. This difference is seen in both early and late effects of radiation on normal tissues. Additionally, different sensitivity of organs leads to different carcinogenesis risks years after exposure [38]. Different effects induced by the bystander effect in different cell types have been observed in in-vitro studies [39-41]. In-vivo studies which have confirmed the expression of carcinogenesis markers in non-targeted tissues is deemed to be different. Tissue specificity in response to the bystander effect is related to the expression of bystander signals in non-irradiated tissues. So, this phenomenon may be independent from radiation sensitivity of organs. The evaluation of DNA methylation changes in non-targeted tissues including the spleen and the skin has indicated distinct specificity at different times. The results showed that local cranial irradiation can cause loss of DNA methylation in the skin only 6 hours after exposure, not later hours. However, cranial irradiation could lead to hypomethylation associated by the inhibition of methyl-binding protein MeCP2 expression in distant spleen tissue for 14 days after exposure [25].

In addition to the role of epigenetic modulators, tissue specificity of bystander effect is related to different expressions of genes involved in reduction/oxidation (redox) system. As mentioned earlier, ROS/NO producing enzymes have a pivotal role in oxidative DNA damage and subsequent genomic instability in bystander cells. Different expressions of genes involved in redox systems which is related to bystander signals, result in different levels of oxidative damage in such tissues. COX-2 is one of the ROS producing enzymes which plays a crucial role in oxidative damage in bystander cells. While COX-2 is not expressed in all types of cells and also the basal level of COX-2 expression is low, in response to bystander signals, the production of this enzyme rises more obviously [42]. In-vivo studies showed that partial irradiation of abdomen leads to increased expression of COX-2 by more than 20-fold in lung and 30-fold in lung bronchial epithelial cells relevant to the control level. The results indicated no significant induction of COX-2 expression and mutagenesis in liver [33, 43].

Carcinogenesis Markers in Non-targeted Cells

Bystander effect is mediated with mechanisms that produces free radicals or change DNA integrity. Role of ROS/NO producing enzymes in oxidative DNA damage in bystander

cells has been confirmed [44, 45]. Moreover, the suppression of antioxidant enzymes aggravates oxidative stress in non-targeted cells [46]. In other words, epigenetic modulators such as microRNAs change the expression of genes involved in cellular processes such as stress response which might increase the risk of carcinogenesis.

Free Radical Production

Ionizing radiation can stimulate different enzymes that produce ROS or NO. Most of these enzymes are immune mediators that kill microorganisms or stimulate inflammatory responses. Upregulation of these inflammatory enzymes such as COX-2, iNOS and NADPH Oxidase is an important marker for increased risk of carcinogenesis [47, 48]. Through inflammatory cytokines such as IL-1, IL-2, IL-8, IL-33 and TNFα and anti-inflammatory cytokines such as TGFβ, the bystander effect stimulates the production of ROS/NO producing enzymes [49]. In addition, a mitochondrial function changes and amplifies oxidative damage induced by inflammatory responses [50, 51]. Continuous free radical production results in oxidative damage to critical targets such as DNA, lipids and proteins. Oxidative damage changes normal functions of these biomolecules that may lead to increased risk of carcinogenesis.

Inhibition of Antioxidant Enzymes

Cells develop both enzymatically and non-enzymatically antioxidant defense systems against detrimental potentials of ROS and RNS. These antioxidant systems consist of low molecular weight such as glutathione (GSH) and antioxidant enzymes such as SOD, CAT and GSH-Px. The suppression of these antioxidant defense systems is one of the most substantial mechanisms involved in radiation toxicity that can result in chronic oxidative damage, genomic instability and interruption of normal structure of tissues [52-54]. Some studies have shown that the inhibition of antioxidant system activity has a key role in the increased ROS levels and the oxidative stress in non-irradiated bystander cells. Najafi et al. exhibited that local irradiation of rat pelvis with 3Gy gamma rays resulted in a remarkable oxidative damage to lung tissue. The results indicated a dramatic decline in SOD activity at 24th and 72nd hours after local irradiation, while direct irradiation increased SOD activity at mentioned times. Furthermore, the levels of GSH in non-targeted lung tissues reduced at the 24th hour after exposure, while direct irradiation did not decrease GSH at the mentioned times [46].

Further researches have proposed the role of epigenetic modulators in the suppression of SOD activity. Epigenetic mediators such as miR-21 affect ROS generation and suppression of SOD activity in bystander cells. Xu et al. revealed that the upregulation of miR-21 is involved in micronuclei formation and 53BP1 foci in bystander cells [55]. Tian and colleagues showed that miR-21 increases ROS production through inhibition of MnSOD [56]. Another study showed that the upregulation of TGF-β is responsible for MnSOD suppression and the increased ROS levels [57]. Targeting TGF-β decreases 53BP1 foci and micronuclei formation in bystander cells [58].

Upregulation of Oncogenes

Oncogenes are genes involved in cell proliferation and are held potential to promote malignancy. These genes are often changed and expressed at abnormal levels in tumor cells. Upregulation of oncogenes through suppression of DNA methylation is considered as a vital mechanism for carcinogenesis effect of bystander effect. It seems that miRNAs play a pivotal role in the expression of DNA methyltransferases (DNMT) genes, especially DNMT3a, DNMT3b, and subsequent hypomethylation [59]. Upregulation or downregulation of some miRNAs can cause changes in the expression of oncogenes through hypomethylation [60]. It is confirmed that miRNAs are involved in the regulation of RAS oncogene and cellular proliferation [61]. Sedelnikova et al. indicated an increased level of DNA

damage associated with loss of nuclear DNA methylation after exposure to radiation [62]. Koturbash et al. showed that local cranial irradiation results in over-expression of miR-194 which leads to inhibition of DNMT3a and methyl-binding protein methyl CpG binding protein 2 [MeCP2] in the distant spleen tissue. These changes contribute to the long term loss of genome DNA methylation that might lead to genomic instability [63].

Genomic Instability

Genomic instability is a phenomenon seen almost in all human cancers. This phenomenon causes chromosomal alterations in cells such as abnormal frequency of mutations within the genome, mutations in tumor suppressor and DNA repair genes, loss of DNA methylation and histone modifications, alteration in mitochondrial DNA and energy balance within cells, attenuation of antioxidant enzymes and so on [64-70]. These changes have been seen in long periods after exposure in direct irradiated and bystander cells [71, 72]. For the first time, Lorimore and colleagues have observed the occurrence of genomic instability markers in the progeny of bystander cells [73]. Seymour et al. showed that the transferred medium from irradiated human epithelial cells can induce genomic instability in the progeny of bystander cells. They investigated that these cells show a reduced plating efficiency [74]. Other studies have shown increased apoptosis, mutations, deletion, abnormal gene amplification and allelic imbalance for both low and high LET radiations [75-77]. It is possible that persistent ROS production, inhibition of antioxidant enzymes and epigenetic changes stimulate continues mutation and induce genomic instability.

The Bystander Effect May Increase Carcinogenesis Risk in Next Generations of Irradiated People

For long years, there have been concerns for heritable effects of ionizing radiation, especially carcinogenesis. Evidence has shown that clastogenic factors induced by ionizing radiation can cause genomic mutation in bystander cells and their offspring. It indicates that genomic mutation induced by bystander effects in germ cells is able to be transferred to next generations. Evidence has demonstrated that genomic instability in germ cells can lead to stable chromosomal abnormalities and be transferred to the second generation [78, 79]. Evidence also reveals that genome instability and epigenetic changes such as altered miRNA expression in the germline cells have an important role in transgenerational effects of radiation. The effects have been considered as upregulation of miR-709, miR-29a and miR-29b, inhibition of DNMT3a and Brother of the Regulator of Imprinted Sites (BORIS), and also obvious hypomethylation of transposable elements such as LINE1 and SINE B2 [80-83].

In an in-vivo study, the bystander effect has been deemed as a mechanism involved in radiation induced genomic instability and transgenerational effects of ionizing radiation. Tamminga et al. showed that local cranial irradiation in rat results in unrepaired DNA damage in the sperm cells and subsequent transgenerational genomic instability, and an obvious epigenetic dysregulation in their progeny. The results additionally indicated a significant hypomethylation in the bone marrow, thymus and spleen of the second generation of exposed animals [84]. These changes are likely to be related to increased risk of carcinogenesis such as hematopoietic malignancies in progeny of irradiated people [85-88].

Conclusion

While the increased survival of patients who have undergone radiotherapy is an incredible success in cancer treatment, there are concerns related to the increased risk of secondary malignancies especially for long years after therapy. Evidence proves that cancer induction in out-of-field organs is the undeniable part of secondary cancers. Studies have admitted the role of the bystander effect in cancer induction

after exposure to radiation. So, attention to this phenomenon is very critical for the evaluation and selection of most appropriated treatment modalities. It seems that the effect of radiation modalities on bystander damage is different compared to directly irradiated cells.

Based on some studies, it seems that DNA damage induced by bystander signaling in non-targeted cells and also its progenies are evaluated less than the irradiation with proton particles compared to lower or higher radiation qualities. It may indicate a beneficial effect of proton therapy compared to x-ray radiotherapy. Interestingly, fractionated irradiation has shown higher damages to bystander cells in comparison with acute single irradiation. Fractionation is a conventional method in radiotherapy to ameliorate the acute effects of exposure to radiation doses which is necessary to kill tumor cells. In contrast to directly irradiated cells, fractionation causes higher toxicity to bystander cells. It possibly reduces the beneficial effects of fractionated radiotherapy. Similar to fractionation, the effect of dose and dose rate on bystander cells are different compared to directly irradiated demonstrate. Some result did not show any effects for different dose rates. Besides, some studies have shown contradictory results suggesting that it might be related to the role of growth factors in bystander cells.

According to different expressions of genes involved in the bystander effect in different cell types, it is predictable that genetic damage and genomic instabilities are different among tissues. It results in different susceptibilities to carcinogenesis in various organs. Furthermore, carcinogenesis susceptibility among non-targeted tissues differs from radiation sensitivity. Genetic mutation in males is different from that in females. Similar to directly irradiated tissues, DNA damage and subsequent consequences are more obvious in males compared to females. However, some studies have shown a higher risk of carcinogenesis after exposure for women compared to men.

Conflict of Interest

None

References

1. Khosroabadi M, Farhood B, Ghorbani M, Hamzian N, Moghaddam HR, Davenport D. Tissue composition effect on dose distribution in neutron brachytherapy/neutron capture therapy. *Rep Pract Oncol Radiother*. 2016;**21**:8-16. doi.org/10.1016/j.rpor.2015.05.001. PubMed PMID: 26900352. PubMed PMCID: 4716403.
2. Gotay CC, Muraoka MY. Quality of life in long-term survivors of adult-onset cancers. *J Natl Cancer Inst*. 1998;**90**:656-67. doi.org/10.1093/jnci/90.9.656. PubMed PMID: 9586662.
3. Narmani A, Farhood B, Haghi-Aminjan H, Mortezazadeh T, Aliasgharzadeh A, Mohseni M, et al. Gadolinium nanoparticles as diagnostic and therapeutic agents: Their delivery systems in magnetic resonance imaging and neutron capture therapy. *J Drug Deliv Sci Technol*. 2018;**44**:457-66.
4. Widel M. Radiation Induced Bystander Effect: From in Vitro Studies to Clinical Application. *International Journal of Medical Physics, Clinical Engineering and Radiation Oncology*. 2016;**5**:1. doi.org/10.4236/ijmpcero.2016.51001.
5. Najafi M, Motevaseli E, Shirazi A, Geraily G, Rezaeyan A, Norouzi F, et al. Mechanisms of inflammatory responses to radiation and normal tissues toxicity: clinical implications. *Int J Radiat Biol*. 2018;**94**(4):335-56.
6. Mancuso M, Pasquali E, Leonardi S, Tanori M, Rebessi S, Di Majo V, et al. Oncogenic bystander radiation effects in Patched heterozygous mouse cerebellum. *Proc Natl Acad Sci U S A*. 2008;**105**:12445-50. doi.org/10.1073/pnas.0804186105. PubMed PMID: 18711141. PubMed PMCID: 2517601.
7. Yahyapour R, Motevaseli E, Rezaeyan A, Abdollahi H, Farhood B, Cheki M, et al. Reduction-oxidation (redox) system in radiation-induced normal tissue injury: molecular mechanisms and implications in radiation therapeutics. *Clin Transl Oncol*. 2018;**20**:975–88. doi:10.1007/s12094-017-1828-6.
8. Bostrom PJ, Soloway MS. Secondary cancer after radiotherapy for prostate cancer: should we be more aware of the risk? *Eur Urol*. 2007;**52**:973-82. doi.org/10.1016/j.eururo.2007.07.002. PubMed PMID: 17644245.
9. Joung JY, Lim J, Oh CM, Jung KW, Cho H, Kim SH, et al. Risk of Second Primary Cancer among Prostate Cancer Patients in Korea: A Population-Based Cohort Study. *PLoS One*. 2015;**10**:e0140693. doi.org/10.1371/journal.pone.0140693. PubMed PMID: 26469085. PubMed PMCID: 4607403.

10. Brenner DJ, Curtis RE, Hall EJ, Ron E. Second malignancies in prostate carcinoma patients after radiotherapy compared with surgery. *Cancer*. 2000;**88**:398-406. doi.org/10.1002/(SICI)1097-0142(20000115)88:2<398::AID-CNCR22>3.0.CO;2-V. PubMed PMID: 10640974.
11. Kleinerman RA, Boice JD, Jr., Storm HH, Sparen P, Andersen A, Pukkala E, et al. Second primary cancer after treatment for cervical cancer. An international cancer registries study. *Cancer*. 1995;**76**:442-52. doi.org/10.1002/1097-0142(19950801)76:3<442::AID-CNCR2820760315>3.0.CO;2-L. PubMed PMID: 8625126.
12. Birgisson H, Pahlman L, Gunnarsson U, Glimelius B. Occurrence of second cancers in patients treated with radiotherapy for rectal cancer. *J Clin Oncol*. 2005;**23**:6126-31. doi.org/10.1200/JCO.2005.02.543. PubMed PMID: 16135478.
13. Bednarz B, Athar B, Xu XG. A comparative study on the risk of second primary cancers in out-of-field organs associated with radiotherapy of localized prostate carcinoma using Monte Carlo-based accelerator and patient models. *Med Phys*. 2010;**37**:1987-94. doi.org/10.1118/1.3367012. PubMed PMID: 20527532. PubMed PMCID: 2862056.
14. Dong C, He M, Ren R, Xie Y, Yuan D, Dang B, et al. Role of the MAPK pathway in the observed bystander effect in lymphocytes co-cultured with macrophages irradiated with gamma-rays or carbon ions. *Life Sci*. 2015;**127**:19-25. doi.org/10.1016/j.lfs.2015.02.017. PubMed PMID: 25748424.
15. Buonanno M, de Toledo SM, Pain D, Azzam EI. Long-term consequences of radiation-induced bystander effects depend on radiation quality and dose and correlate with oxidative stress. *Radiat Res*. 2011;**175**:405-15. doi.org/10.1667/RR2461.1. PubMed PMID: 21319986. PubMed PMCID: 3106980.
16. Autsavapromporn N, Suzuki M, Funayama T, Usami N, Plante I, Yokota Y, et al. Gap junction communication and the propagation of bystander effects induced by microbeam irradiation in human fibroblast cultures: the impact of radiation quality. *Radiat Res*. 2013;**180**:367-75. doi.org/10.1667/RR3111.1. PubMed PMID: 23987132. PubMed PMCID: 4058832.
17. Anzenberg V, Chandiramani S, Coderre JA. LET-dependent bystander effects caused by irradiation of human prostate carcinoma cells with X rays or alpha particles. *Radiat Res*. 2008;**170**:467-76. doi.org/10.1667/RR1312.1. PubMed PMID: 19024654. PubMed PMCID: 4132631.
18. Autsavapromporn N, Plante I, Liu C, Konishi T, Usami N, Funayama T, et al. Genetic changes in progeny of bystander human fibroblasts after microbeam irradiation with X-rays, protons or carbon ions: the relevance to cancer risk. *Int J Radiat Biol*. 2015;**91**:62-70. doi.org/10.3109/09553002.2014.950715. PubMed PMID: 25084840.
19. Chen HH, Jia RF, Yu L, Zhao MJ, Shao CL, Cheng WY. Bystander effects induced by continuous low-dose-rate 125I seeds potentiate the killing action of irradiation on human lung cancer cells in vitro. *Int J Radiat Oncol Biol Phys*. 2008;**72**:1560-6. doi.org/10.1016/j.ijrobp.2008.07.038. PubMed PMID: 19028278.
20. Liu Z, Mothersill CE, McNeill FE, Lyng FM, Byun SH, Seymour CB, et al. A dose threshold for a medium transfer bystander effect for a human skin cell line. *Radiat Res*. 2006;**166**:19-23. doi.org/10.1667/RR3580.1. PubMed PMID: 16808607.
21. Gow MD, Seymour CB, Byun SH, Mothersill CE. Effect of dose rate on the radiation-induced bystander response. *Phys Med Biol*. 2008;**53**:119-32. doi.org/10.1088/0031-9155/53/1/008. PubMed PMID: 18182691.
22. Mothersill C, Seymour CB. Bystander and delayed effects after fractionated radiation exposure. *Radiat Res*. 2002;**158**:626-33. doi.org/10.1667/0033-7587(2002)158[0626:BADEAF]2.0.CO;2. PubMed PMID: 12385640.
23. Soleymanifard S, Toossi MT, Samani RK, Mohebbi S. Investigation of the bystander effect in MRC5 cells after acute and fractionated irradiation in vitro. *J Med Phys*. 2014;**39**:93-7. doi.org/10.4103/0971-6203.131282. PubMed PMID: 24872606. PubMed PMCID: 4035621.
24. Soleymanifard S, Bahreyni Toossi MT, Kamran Samani R, Mohebbi S. Comparison of Radiation-Induced Bystander Effect in QU-DB Cells after Acute and Fractionated Irradiation: An In Vitro Study. *Cell J*. 2016;**18**:346-52. PubMed PMID: 27602316. PubMed PMCID: 5011322.
25. Ilnytskyy Y, Koturbash I, Kovalchuk O. Radiation-induced bystander effects in vivo are epigenetically regulated in a tissue-specific manner. *Environ Mol Mutagen*. 2009;**50**:105-13. doi.org/10.1002/em.20440. PubMed PMID: 19107897.
26. Yahyapour R, Amini P, Rezapour S, Cheki M, Rezaeyan A, Farhood B, et al. Radiation-induced inflammation and autoimmune diseases. *Mil Med Res*. 2018;**5**(1):9.
27. Guizard AV, Boutou O, Pottier D, Troussard X, Pheby D, Launoy G, et al. The incidence of childhood leukaemia around the La Hague nuclear waste reprocessing plant (France): a survey for the years 1978-1998. *J Epidemiol Community Health*. 2001;**55**:469-74. doi.org/10.1136/jech.55.7.469. PubMed PMID: 11413175. PubMed PMCID: 1731936.
28. Yoshida K, Nemoto K, Nishimura M, Seki M. Exacerbating factors of radiation-induced myeloid leukemogenesis. *Leuk Res*. 1993;**17**:437-40. doi.org/10.1016/0145-2126(93)90099-7. PubMed PMID: 8501971.

29. Kovalchuk O, Burke P, Besplug J, Slovack M, Filkowski J, Pogribny I. Methylation changes in muscle and liver tissues of male and female mice exposed to acute and chronic low-dose X-ray-irradiation. *Mutat Res*. 2004;**548**:75-84. doi.org/10.1016/j.mrfmmm.2003.12.016. PubMed PMID: 15063138.
30. Koturbash I, Zemp F, Kolb B, Kovalchuk O. Sex-specific radiation-induced microRNAome responses in the hippocampus, cerebellum and frontal cortex in a mouse model. *Mutat Res*. 2011;**722**:114-8. doi.org/10.1016/j.mrgentox.2010.05.007. PubMed PMID: 20478395.
31. Koturbash I, Kutanzi K, Hendrickson K, Rodriguez-Juarez R, Kogosov D, Kovalchuk O. Radiation-induced bystander effects in vivo are sex specific. *Mutat Res*. 2008;**642**:28-36. doi.org/10.1016/j.mrfmmm.2008.04.002. PubMed PMID: 18508093.
32. Koturbash I, Zemp FJ, Kutanzi K, Luzhna L, Loree J, Kolb B, et al. Sex-specific microRNAome deregulation in the shielded bystander spleen of cranially exposed mice. *Cell Cycle*. 2008;**7**:1658-67. doi.org/10.4161/cc.7.11.5981. PubMed PMID: 18560276.
33. Chai Y, Calaf GM, Zhou H, Ghandhi SA, Elliston CD, Wen G, et al. Radiation induced COX-2 expression and mutagenesis at non-targeted lung tissues of gpt delta transgenic mice. *Br J Cancer*. 2013;**108**(1):91-8. PubMed PMID: 23321513. PubMed PMCID: 3553512.
34. Fardid R, Najafi M, Salajegheh A, Kazemi E, Rezaeyan A. Radiation-induced non-targeted effect in vivo: Evaluation of cyclooygenase-2 and endothelin-1 gene expression in rat heart tissues. *J Cancer Res Ther*. 2017;**13**:51-5. doi.org/10.4103/0973-1482.203601. PubMed PMID: 28508833.
35. Friedman DL, Whitton J, Leisenring W, Mertens AC, Hammond S, Stovall M, et al. Subsequent neoplasms in 5-year survivors of childhood cancer: the Childhood Cancer Survivor Study. *J Natl Cancer Inst*. 2010;**102**:1083-95. doi.org/10.1093/jnci/djq238. PubMed PMID: 20634481. PubMed PMCID: 2907408.
36. Armstrong GT, Sklar CA, Hudson MM, Robison LL. Long-term health status among survivors of childhood cancer: does sex matter? *J Clin Oncol*. 2007;**25**:4477-89. doi.org/10.1200/JCO.2007.11.2003. PubMed PMID: 17906209.
37. Taddei PJ, Mahajan A, Mirkovic D, Zhang R, Giebeler A, Kornguth D, et al. Predicted risks of second malignant neoplasm incidence and mortality due to secondary neutrons in a girl and boy receiving proton craniospinal irradiation. *Phys Med Biol*. 2010;**55**:7067-80. doi.org/10.1088/0031-9155/55/23/S08. PubMed PMID: 21076189. PubMed PMCID: 3001324.
38. Valentin J. The 2007 recommendations of the international commission on radiological protection. Oxford: Elsevier; 2007.
39. Toossi MT, Mohebbi S, Samani RK, Soleymanifard S. MRC5 and QU-DB bystander cells can produce bystander factors and induce radiation bystander effect. *J Med Phys*. 2014;**39**:192-6. doi.org/10.4103/0971-6203.139011. PubMed PMID: 25190998. PubMed PMCID: 4154187.
40. Soleymanifard S, Bahreyni Toossi MT, Sazgarnia A, Mohebbi S. The role of target and bystander cells in dose-response relationship of radiation-induced bystander effects in two cell lines. *Iran J Basic Med Sci*. 2013;**16**:177-83. PubMed PMID: 24298387. PubMed PMCID: 3843862.
41. Soleymanifard S, Bahreyni MT. Comparing the level of bystander effect in a couple of tumor and normal cell lines. *J Med Phys*. 2012;**37**:102-6. doi.org/10.4103/0971-6203.94745. PubMed PMID: 22557800. PubMed PMCID: 3339141.
42. Cheki M, Yahyapour R, Farhood B, Rezaeyan A, Shabeeb D, Amini P, et al. COX-2 in Radiotherapy: A Potential Target for Radioprotection and Radiosensitization. Curr Mol Pharmacol. 2018;11:173–83. doi: 10.2174/1874467211666180219102520.
43. Chai Y, Lam RK, Calaf GM, Zhou H, Amundson S, Hei TK. Radiation-induced non-targeted response in vivo: role of the TGFbeta-TGFBR1-COX-2 signalling pathway. *Br J Cancer*. 2013;**108**:1106-12. doi.org/10.1038/bjc.2013.53. PubMed PMID: 23412109. PubMed PMCID: 3619070.
44. Bishayee A, Hill HZ, Stein D, Rao DV, Howell RW. Free radical-initiated and gap junction-mediated bystander effect due to nonuniform distribution of incorporated radioactivity in a three-dimensional tissue culture model. *Radiat Res*. 2001;**155**:335-44. doi.org/10.1667/0033-7587(2001)155[0335:FRIAGJ]2.0.CO;2. PubMed PMID: 11175669. PubMed PMCID: 3495610.
45. Yakovlev VA. Role of nitric oxide in the radiation-induced bystander effect. *Redox Biol*. 2015;**6**:396-400. doi.org/10.1016/j.redox.2015.08.018. PubMed PMID: 26355395. PubMed PMCID: 4572387.
46. Najafi M, Fardid R, Takhshid MA, Mosleh-Shirazi MA, Rezaeyan AH, Salajegheh A. Radiation-Induced Oxidative Stress at Out-of-Field Lung Tissues after Pelvis Irradiation in Rats. *Cell J*. 2016;**18**:340-5. PubMed PMID: 27602315. PubMed PMCID: 5011321.
47. Najafi M, Shirazi A, Motevaseli E, Geraily G, Norouzi F, Heidari M, et al. The melatonin immunomodulatory actions in radiotherapy. *Biophys Rev*. 2017;**9**:139-48. doi.org/10.1007/s12551-017-0256-8. PubMed PMID: 28510090. PubMed PMCID: 5425818.
48. Fardid R, Salajegheh A, Mosleh-Shirazi MA, Sharifzadeh S, Okhovat MA, Najafi M, et al. Melatonin Ameliorates The Production of COX-2, iNOS, and The Formation of 8-OHdG in Non-Targeted Lung Tissue after Pelvic Irradiation. *Cell J*. 2017;**19**:324-31. PubMed PMID: 28670525. PubMed PMCID:

5412791.

49. Yahyapour R, Motevaseli E, Rezaeyan A, Abdollahi H, Farhood B, Cheki M, et al. Mechanisms of Radiation Bystander and Non-Targeted Effects: Implications to Radiation Carcinogenesis and Radiotherapy. *Curr Radiopharm*. 2018;**11**:34–45. doi: 10.2174/1874471011666171229123130.
50. Rajendran S, Harrison SH, Thomas RA, Tucker JD. The role of mitochondria in the radiation-induced bystander effect in human lymphoblastoid cells. *Radiat Res*. 2011;**175**:159-71. doi.org/10.1667/RR2296.1. PubMed PMID: 21268709.
51. Yang G, Wu L, Chen S, Zhu L, Huang P, Tong L, et al. Mitochondrial dysfunction resulting from loss of cytochrome c impairs radiation-induced bystander effect. *Br J Cancer*. 2009;**100**:1912-6. doi.org/10.1038/sj.bjc.6605087. PubMed PMID: 19455142. PubMed PMCID: 2714242.
52. Haddadi GH, Rezaeyan A, Mosleh-Shirazi MA, Hosseinzadeh M, Fardid R, Najafi M, et al. Hesperidin as Radioprotector against Radiation-induced Lung Damage in Rat: A Histopathological Study. *J Med Phys*. 2017;**42**:25-32. doi.org/10.4103/jmp.JMP_119_16. PubMed PMID: 28405105. PubMed PMCID: 5370335.
53. Rezaeyan A, Haddadi GH, Hosseinzadeh M, Moradi M, Najafi M. Radioprotective effects of hesperidin on oxidative damages and histopathological changes induced by X-irradiation in rats heart tissue. *J Med Phys*. 2016;**41**:182-91. doi.org/10.4103/0971-6203.189482. PubMed PMID: 27651565. PubMed PMCID: 5019037.
54. Irshad M, Chaudhuri PS. Oxidant-antioxidant system: role and significance in human body. *Indian J Exp Biol*. 2002;**40**:1233-9. PubMed PMID: 13677624.
55. Xu S, Ding N, Pei H, Hu W, Wei W, Zhang X, et al. MiR-21 is involved in radiation-induced bystander effects. *RNA Biol*. 2014;**11**:1161-70. doi.org/10.4161/rna.34380. PubMed PMID: 25483031. PubMed PMCID: 4615763.
56. Tian W, Yin X, Wang L, Wang J, Zhu W, Cao J, et al. The key role of miR-21-regulated SOD2 in the medium-mediated bystander responses in human fibroblasts induced by alpha-irradiated keratinocytes. *Mutat Res*. 2015;**780**:77-85. doi.org/10.1016/j.mrfmmm.2015.08.003. PubMed PMID: 26302379.
57. Jiang Y, Chen X, Tian W, Yin X, Wang J, Yang H. The role of TGF-beta1-miR-21-ROS pathway in bystander responses induced by irradiated non-small-cell lung cancer cells. *Br J Cancer*. 2014;**111**:772-80. doi.org/10.1038/bjc.2014.368. PubMed PMID: 24992582. PubMed PMCID: 4134503.
58. Hu W, Xu S, Yao B, Hong M, Wu X, Pei H, et al. MiR-663 inhibits radiation-induced bystander effects by targeting TGFB1 in a feedback mode. *RNA Biol*. 2014;**11**:1189-98. doi.org/10.4161/rna.34345. PubMed PMID: 25483041. PubMed PMCID: 4615905.
59. Benetti R, Gonzalo S, Jaco I, Munoz P, Gonzalez S, Schoeftner S, et al. A mammalian microRNA cluster controls DNA methylation and telomere recombination via Rbl2-dependent regulation of DNA methyltransferases. *Nat Struct Mol Biol*. 2008;**15**:268-79. doi.org/10.1038/nsmb.1399. PubMed PMID: 18311151. PubMed PMCID: 2990406.
60. Das PM, Singal R. DNA methylation and cancer. *J Clin Oncol*. 2004;**22**:4632-42. doi.org/10.1200/JCO.2004.07.151. PubMed PMID: 15542813.
61. Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer*. 2006;**94**:776-80. doi.org/10.1038/sj.bjc.6603023. PubMed PMID: 16495913. PubMed PMCID: 2361377.
62. Sedelnikova OA, Nakamura A, Kovalchuk O, Koturbash I, Mitchell SA, Marino SA, et al. DNA double-strand breaks form in bystander cells after microbeam irradiation of three-dimensional human tissue models. *Cancer Res*. 2007;**67**:4295-302. doi.org/10.1158/0008-5472.CAN-06-4442. PubMed PMID: 17483342.
63. Koturbash I, Boyko A, Rodriguez-Juarez R, McDonald RJ, Tryndyak VP, Kovalchuk I, et al. Role of epigenetic effectors in maintenance of the long-term persistent bystander effect in spleen in vivo. *Carcinogenesis*. 2007;**28**:1831-8. doi.org/10.1093/carcin/bgm053. PubMed PMID: 17347136.
64. Najafi M, Cheki M, Rezapoor S, Geraily G, Motevaseli E, Carnovale C, Clementi E. Metformin: Prevention of genomic instability and cancer: *A review. Mutat Res* . 2018;**827**:1–8. doi: 10.1016/j.mrgentox.2018.01.007.
65. Ferguson LR, Chen H, Collins AR, Connell M, Damia G, Dasgupta S, et al. Genomic instability in human cancer: Molecular insights and opportunities for therapeutic attack and prevention through diet and nutrition. *Semin Cancer Biol*. 2015;**35**:S5-s24. doi.org/10.1016/j.semcancer.2015.03.005. PubMed PMID: 25869442. PubMed PMCID: 4600419.
66. Najafi M, Shirazi A, Motevaseli E, Rezaeyan A, Salajegheh A, Rezapoor S. Melatonin as an anti-inflammatory agent in radiotherapy. *Inflammopharmacology*. 2017;**25**:403–13. doi: 10.1007/s10787-017-0332-5.
67. Chatterjee A, Dasgupta S, Sidransky D. Mitochondrial subversion in cancer. *Cancer Prev Res* (*Phila*). 2011;**4**:638-54. doi.org/10.1158/1940-6207.CAPR-10-0326. PubMed PMID: 21543342. PubMed PMCID: 3298745.
68. Yahyapour R, Amini P, Rezapoor S, Rezaeyan A, Farhood B, Cheki M, Fallah H. Targeting of Inflammation for Radiation Protection and Mitigation. *Curr Mol Pharmacol*. 2018;**11**:203–10. doi: 10.2174/1874467210666171108165641.
69. Konki M, Pasumarthy K, Malonzo M, Sainio A,

Valensisi C, Soderstrom M, et al. Epigenetic Silencing of the Key Antioxidant Enzyme Catalase in Karyotypically Abnormal Human Pluripotent Stem Cells. *Sci Rep*. 2016;**6**:22190. doi.org/10.1038/srep22190. PubMed PMID: 26911679. PubMed PMCID: 4766493.

70. Rooney S, Alt FW, Lombard D, Whitlow S, Eckersdorff M, Fleming J, et al. Defective DNA repair and increased genomic instability in Artemis-deficient murine cells. *J Exp Med*. 2003;**197**:553-65. doi.org/10.1084/jem.20021891. PubMed PMID: 12615897. PubMed PMCID: 2193825.
71. Lorimore SA, Coates PJ, Wright EG. Radiation-induced genomic instability and bystander effects: inter-related nontargeted effects of exposure to ionizing radiation. *Oncogene*. 2003;**22**:7058-69. doi.org/10.1038/sj.onc.1207044. PubMed PMID: 14557811.
72. Morgan WF, Day JP, Kaplan MI, McGhee EM, Limoli CL. Genomic instability induced by ionizing radiation. *Radiat Res*. 1996;**146**:247-58. doi.org/10.2307/3579454. PubMed PMID: 8752302.
73. Lorimore SA, Kadhim MA, Pocock DA, Papworth D, Stevens DL, Goodhead DT, et al. Chromosomal instability in the descendants of unirradiated surviving cells after alpha-particle irradiation. *Proc Natl Acad Sci U S A*. 1998;**95**:5730-3. doi.org/10.1073/pnas.95.10.5730. PubMed PMID: 9576952. PubMed PMCID: 20447.
74. Seymour CB, Mothersill C. Delayed expression of lethal mutations and genomic instability in the progeny of human epithelial cells that survived in a bystander-killing environment. *Radiat Oncol Investig*. 1997;**5**:106-10. doi.org/10.1002/(SICI)1520-6823(1997)5:3<106::AID-ROI4>3.0.CO;2-1. PubMed PMID: 9303065.
75. Little JB, Nagasawa H, Pfenning T, Vetrovs H. Radiation-induced genomic instability: delayed mutagenic and cytogenetic effects of X rays and alpha particles. *Radiat Res*. 1997;**148**:299-307. doi.org/10.2307/3579514. PubMed PMID: 9339945.
76. Lyng FM, Seymour CB, Mothersill C. Initiation of apoptosis in cells exposed to medium from the progeny of irradiated cells: a possible mechanism for bystander-induced genomic instability? *Radiat Res*. 2002;**157**:365-70. doi.org/10.1667/0033-7587(2002)157[0365:IOAICE]2.0.CO;2. PubMed PMID: 11893237.
77. Hall EJ, Hei TK. Genomic instability and bystander effects induced by high-LET radiation. *Oncogene*. 2003;**22**:7034-42. doi.org/10.1038/sj.onc.1206900. PubMed PMID: 14557808.
78. Niwa O. Induced genomic instability in irradiated germ cells and in the offspring; reconciling discrepancies among the human and animal studies. *Oncogene*. 2003;**22**:7078-86. doi.org/10.1038/sj.onc.1207037. PubMed PMID: 14557813.
79. Salimi M, Mozdarani H, Nazari E. Cytogenetic Alterations in Preimplantation Mice Embryos Following Male Mouse Gonadal Gamma-irradiation: Comparison of Two Methods for Reproductive Toxicity Screening. *Avicenna J Med Biotechnol*. 2014;**6**:130-9. PubMed PMID: 25215176. PubMed PMCID: 4147099.
80. Filkowski JN, Ilnytskyy Y, Tamminga J, Koturbash I, Golubov A, Bagnyukova T, et al. Hypomethylation and genome instability in the germline of exposed parents and their progeny is associated with altered miRNA expression. *Carcinogenesis*. 2010;**31**:1110-5. doi.org/10.1093/carcin/bgp300. PubMed PMID: 19959559.
81. Koturbash I, Baker M, Loree J, Kutanzi K, Hudson D, Pogribny I, et al. Epigenetic dysregulation underlies radiation-induced transgenerational genome instability in vivo. *Int J Radiat Oncol Biol Phys*. 2006;**66**:327-30. doi.org/10.1016/j.ijrobp.2006.06.012. PubMed PMID: 16965987.
82. Tamminga J, Kovalchuk O. Role of DNA damage and epigenetic DNA methylation changes in radiation-induced genomic instability and bystander effects in germline in vivo. *Curr Mol Pharmacol*. 2011;**4**:115-25. doi.org/10.2174/1874467211104020115. PubMed PMID: 21143184.
83. Tamminga J, Kathiria P, Koturbash I, Kovalchuk O. DNA damage-induced upregulation of miR-709 in the germline downregulates BORIS to counteract aberrant DNA hypomethylation. *Cell Cycle*. 2008;**7**:3731-6. doi.org/10.4161/cc.7.23.7186. PubMed PMID: 19029807.
84. Tamminga J, Koturbash I, Baker M, Kutanzi K, Kathiria P, Pogribny IP, et al. Paternal cranial irradiation induces distant bystander DNA damage in the germline and leads to epigenetic alterations in the offspring. *Cell Cycle*. 2008;**7**:1238-45. doi.org/10.4161/cc.7.9.5806. PubMed PMID: 18418050.
85. Luke GA, Riches AC, Bryant PE. Genomic instability in haematopoietic cells of F1 generation mice of irradiated male parents. *Mutagenesis*. 1997;**12**:147-52. doi.org/10.1093/mutage/12.3.147. PubMed PMID: 9175639.
86. Lord BI. Transgenerational susceptibility to leukaemia induction resulting from preconception, paternal irradiation. *Int J Radiat Biol*. 1999;75:801-10. doi.org/10.1080/095530099139854. PubMed PMID: 10489891.
87. Gardner MJ. Leukemia in children and paternal radiation exposure at the Sellafield nuclear site. *J Natl Cancer Inst Monogr*. 1992;(12):133-5. PubMed PMID: 1616797.
88. Najafi M, Salajegheh A, Rezaeyan A. Bystander Effect and Second Primary Cancers following Radiotherapy: What are its Significances? *J Med Phys*. 2017;**42**:55-6. doi.org/10.4103/jmp.JMP_124_16. PubMed PMID: 28405109. PubMed PMCID: 5370339.