

# The Mechanisms of Radiation-Induced Bystander Effect

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## ABSTRACT

The radiation-induced bystander effect is the phenomenon which non-irradiated cells exhibit effects along with their different levels as a result of signals received from nearby irradiated cells. Responses of non-irradiated cells may include changes in process of translation, gene expression, cell proliferation, apoptosis and cells death. These changes are confirmed by results of some In-Vivo studies. Most well-known important factors affecting radiation-induced bystander effect include free radicals, immune system factors, expression changes of some genes involved in inflammation pathway and epigenetic factors.

## Keywords

Bystander effect, Immune system, Free radicals, Mitochondria, Epigenetic

## Introduction

Traditionally it was believed in radiobiology that only direct radiation exposure to genetic material of nucleus causes damage to cells. This model predicts that radiation-induced mutations are created in irradiated area during a short time after irradiation. This dogma was challenged in 1992 with an experiment performed by Nagasawa. This experiment revealed that irradiation of 1% cells with alpha particles lead to chromatid exchange in more than 30% of cells. This is called as bystander effect which demonstrates the relationship between irradiated and non-irradiated cells [1]. Results of studies on survivors of Chernobyl explosion as well as radiotherapy of patients with cancer revealed that adding blood serum of these individuals to the same non-irradiated cell culture causes chromosomal damage [2]. These effects are persistent and would remain even in whom had a radiation exposure twenty years before [3]. It is proposed that irradiated people blood has clastogenic factors. Bystander effect is more obvious in cells with gap junction. Therefore, intercellular relationships between cells is an necessary requirement to transfer signals of radiation-induced bystander effect[4].

Distant Bystander effect is proved to be existed outside of radiation field according to in-Vivo studies. Local irradiation to a small area of body causes chromosomal damages and changes in the cell and molecule levels of distant tissues. After a local irradiation, the chromosomal breaks, P53 activity, DNA repair enzymes, mitotic death and apoptosis of distant tissues from radiation target would impressively increased[5]. These signs are a threat for carcinogenesis due to radiation induced by-

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stander effect. Mancuso and their teammates found that irradiated mice with high sensitivity increase induced medulloblastoma cancer associated with chromosomal damages and abnormalities apparently. Their study proved carcinogenesis of the bystander effect directly [6].

It is supposed that local radiation to an area, like what is seen in radiotherapy, could cause systemic damages and even lead to carcinogenesis incidence beyond therapy field. An example of secondary cancer which is attributed to this phenomenon is high incidence of lung cancer among people who have had radiation therapy to treat prostate cancer [7, 8]. Mechanisms involved in establishing the bystander effect or the radiation effect of outside therapy field include immune system, Free radicals, oxidative stress, changes in gene expression of inflammation pathway and epigenetic modulators.

### Mechanisms involved in the bystander effect

#### Immune system

The immune system consists of various types of molecules which protect body against infection and cellular damages. Immune system applies its effects through molecules called cytokines. Cytokines could change the secretion of some molecules as well as cell proliferation by affecting expression or membrane proteins inhibition. Therefore cytokines have effect on regulation of immune response, inflammation and proliferation of blood cells. The most important immune system factors involved in radiation-induced bystander effect are lymphocytes and macrophages [9]. Ionizing radiation by stimulating these cells elevates the level of most cytokines such as IL-1, IL-2, IL-6, IL-8, TNF $\alpha$  and TGF $\beta$  in non-irradiated cells. Partial irradiation of the lung demonstrated the increases of these cytokines in the shielded lung area [10]. Elevation of these cytokines plays a key role in second malignancy after

radiotherapy[11]. Most of these cytokines are involved in proliferation and differentiation of stem cells[12]. Tumor Necrosis Factor Alpha (TNF $\alpha$ ) which is increased evidently after acute exposure [13] leads to induce necrosis and cell death in tumor cells, however, this is not happened to normal cells. TNF $\alpha$  along with other stated cytokines are placed in the inflammation pathway causes nitric oxide production. Activated macrophages by increasing cytokines production lead to increased chromosomal damages, change in DNA bases, mutagenesis and apoptosis in non-irradiated cells. Increasing production level of cytokines via macrophages stimulates NO production which leads to oxidative stress. Superoxide anions are known as an important mediator for damages of clastogenic factors[14].

#### Free radicals

Free radicals have a too short life time which causes their inability to reach other cells after being produced in the cells by the collision of radiation with water molecules. Therefore, free radicals are not considered as a factor in the damage of non-irradiated cells. Free radicals in the presence of oxygen can be converted to long-lived peroxides. Studies using electron spin shows that these peroxides can have over twenty hours half-life [15]. Although half-life of most peroxides makes them be lesser reactive than free radicals, but this helps them to pass longer paths within or outside the cell. Thus peroxides through this can cause damage to cells which are not exposed to radiation. Many In-Vitro experiments have indicated that free radical and peroxides scavengers such as DMSO and vitamin C reduce chromosomal damage such as chromosomal breaks, apoptosis and micronuclei [16, 17]. These results suggest that production of free radicals after irradiation plays a significant role in chromosomal damage of non-irradiated cells, however, even using free radical scavengers cannot completely suppress these damages, thus it reflects the role of other factors in

the establishment of the bystander effect [17].

Mitochondrial activity has a serious role in development of the bystander effect through the production of free radicals. Studies represent that chain suppression of mitochondrial cellular respiratory, calcium uptake with mitochondria and also depleting the cells out of mtDNA, would decrease the chromosomal damage significantly [18]. A malfunction in cytochrome c which is located on the inner wall of mitochondria, and involved in the chain of electron transport illustrated similar results[19]. Other studies also confirmed that mitochondria can increase the cellular damage caused by bystander effect through increase in production of superoxide and free radicals, [20, 21].

Another factor affecting the production of free radicals in cells is NADPH Oxidase. NADPH Oxidase is an enzyme which is dependent on cell membrane and can has the ability to generate free radicals continually leads to cellular and chromosomal damage. Suppressing this enzyme after total body radiation has proved to be effective on the reduction of radiation effects on hematopoietic cells [22]. NADPH Oxidase gene expression may be increased by the influence of protein kinase c and p38 [23]. Studies on fibroblasts and epithelial cell lines suggest that this enzyme by production of free radicals has a key role in inducing the bystander effect [24].

#### Altering gene expression

Genes which are involved in the inducing of the bystander effect are often the same genes involved in inflammatory pathways. The most important types of these genes are mitogen activated protein kinases (MAPKs), nuclear factor of kappa B (NFkB), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Increase of these genes Expression which occurs by various factors leads to inflammation and NO production which consequently elevates oxidative stress[14]. As it is above explained, radiation exposure induce

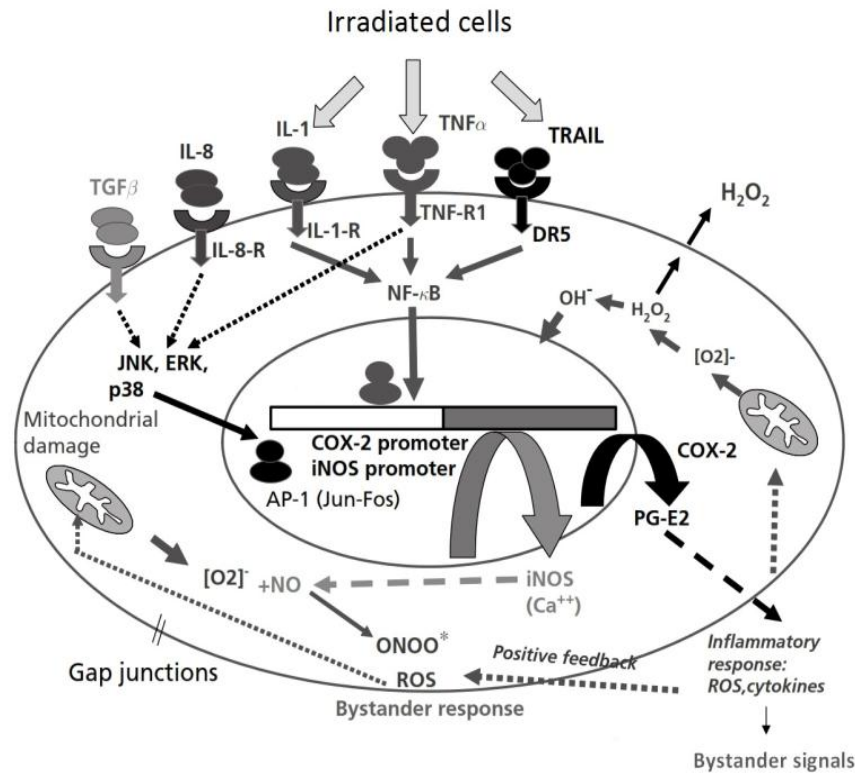
macrophages to produce cytokines such as IL-1, IL-2, IL-8, TGF $\beta$  and TNF $\alpha$ . Receptors of these cytokines which are located on the cell surface are stimulated by these factors and alter a cascade of genes expression. Such alterations among tissues which are exposed to direct irradiation are the main factor of the tissues inflammation [25].

Considered cytokines through stimulating the expression of NFkB gene or MAPK genes such as ERK, JUN and P38 genes causes transcription activation of cyclooxygenase-2 and iNOS. Cyclooxygenase-2 is not expressed in all tissues, besides its expression level is too low. Hence, a small increase in the expression of this gene is too obvious. Cyclooxygenase-2 is the main factor in producing prostaglandins like PG-E2 and PG-I2 which induces the blood vessels dilatation as well as incidence of inflammation symptoms [26, 27]. iNOS also produces nitric oxide and thereby increases the level of oxidative stress. Over expression of this gene is often associated with an increase in cyclooxygenase-2 [28].

According to above mentioned, it is expected that acute doses of radiation through stimulating macrophages activities, causes the production of cytokines which leads to increase the expression of COX-2 and iNOS genes in non-irradiated cells. In-Vitro Studies have shown that the bystander effect can have a threefold increase of cyclooxygenase-2 in nearby non-irradiated cells [27]. In-vivo study also has shown multi fold increase in the expression of this gene during 72 hours after radiation of lung[29, 30]. (figure 1)

#### Epigenetic modulators

Epigenetic term was used by Conrad Waddington for the first time in 1942 to explain the effect of environment on genes which create a new phenotype [31]. Today, epigenetic includes the changes in the genome without any change in DNA sequence. Epigenetic is a system which makes changes in gene expression by mediation of molecules which bind to



**Figure 1:** The mechanisms of radiation-induced bystander effect with alteration the expression of involved genes in the inflammation pathway

chromosomes. These combinations include the groups of methyl, acetyl, phosphor, ubiquitinae and molecules such as miRNA, siRNA and piRNA. The methyl group plays the most important role in the changes of genes expression. Adding methyl groups to chromosomes is called methylation which reduces the transcription proteins binding to chromosome and subsequently decreases gene expression. Besides, reduction of methyl groups in the promoter region of genes increases gene expression[32]. Such these changes (Methylation and demethylation) can be transmitted to the future generations [33].

CpG islands are located in the regions of genes promoter and connect to particular molecules (initial proteins) which are familiar with these regions and make the genes to be

expressed.. In somatic cells, approximately 70% of CpG zones in the human genome are naturally methylated, which contributes the suppression of numerous genes expressions. [33]. When chromosome regions are in an open position, CpG islands relatively become demethylated to increase access to gene promoters. By contrast, the connection of this group to cytosine base makes less exposure of DNA strand to expression (hypermethylation), as the result, these changes makes the gene to be turned off [34]. Hypomethylation by increasing of oncogenes activity and hypermethylation by decreasing of tumor suppressor genes both involved in carcinogenesis. Loosing methylation among denucleotides of CPG is the first unnatural sign of cancerous cells which increases DNA breaks, anipolo-

sis, mutation increase and consequently the phenomenon of genomic instability [34].

In mammals, there are three types of DNA methyltransferase which are mainly responsible for setting and establishing methyl patterns in CPG regions including DNA methyltransferase 1, methyltransferase 3B, and methyltransferase 3A. Among these enzymes, DNA methyltransferase 1 is the main enzyme in the methylation patterns after DNA replication [35]. Generally, it can be said that epigenetic alterations are permanent mutagenesis changes which mainly include DNA methylation and histone modifications.

miRNA, siRNA and piRNA are molecules which their bind to genes and turns off genes expression. These molecules can get out from the origin tissue and alter gene expression in distant tissues [36]. Decrease and increase of miRNA along with increase of oncogenes or decrease of tumor suppressor genes expression are contributed factors of carcinogenesis [37]. Hence, non-coding RNAs play role in epigenetic alterations by reduction of genes expression. Other epigenetic alterations include changes in proteins such as histones which bind to DNA to shape it as a specific structure [38].

Epigenetic factors can significantly affected by direct irradiation. It is demonstrated that radiation can affect methylation patterns. Acute exposures to radiation of x or gamma-ray can lead to a global hypomethylation. It is proved that radiation may change the methylation level among directly irradiated tissues. Observed hypomethylation is related to DNA modification after radiation. This phenomenon is associated with induced changes in the expression of DNA methyltransferases especially DNMT3a and DNMT3b methyltransferases which are affected by irradiation. The most important change which may be affected by this phenomenon is hypomethylation which plays a key role in genomic instability and mutagenesis [39, 40].

Bystander effect may be effective in lots of genetic alterations, including chromosomal abnormalities, sister chromatid exchanges, deletions, transcriptions, mutations and gene expressions. In-Vitro studies suggest that the bystander effect of radiation causes irregularity in the level of chromosome methylation which leads to increase in genomic instability, chromatid and chromosome abnormalities, apoptosis and cell death [5].

Some studies have been continued by 3D phantoms. These studies showed a significant increase in H2AX phosphorylations, a reduction in DNA methylation levels and an increase in chromosome breaks among non-irradiated cells. There was a significant increase in the amount of H2AX histone phosphorylations among non-irradiated tissues. In the long-term the rate of apoptosis and micronucleus increased, DNA methylation of nuclear decreased then the growth was stopped and a rise in the damage of sensitive cell were observed. The considerable note under these studies is losing methylation among non-irradiated cells. Reduction of DNA methylation which relates to other epigenetic factors illustrates the effects of epigenetic factors in inducing of radiation-induced bystander effect [41].

It is shown by analyses that a number miRNAs, particularly let-7 family play an important role in the cellular response to oxidative stress conditions. These miRNAs are important in regulation of RAS genes expressions and cellular proliferation. Direct radiation exposure reduces expressions of these miRNAs obviously [42]. Small miRNAs are involved in many of cellular processes such as cell-cell communication. They are small in size, relatively stable, and able to pass through the cells long distances in the body. Thus these molecules are good candidates for inducing of radiation-induced bystander effect.

Radiation exposure leads to over-expression of miR-29, miR-16 and miR-17. MiR-29 reduces the expression of the DNA methyltrans-

ferase 3A and MCL1 which is an important factor of methylation and apoptosis. MiR-16 is effective in altering expression of BCL-2. This protein also plays a significant role in the regulation of apoptosis. Over-expression of miR-17 is associated with reduction of E2F1 and RB1. These two genes contribute to regulate the cell cycle especially in the passing of cell cycle from G1 phase to S phase and to induce apoptosis. These results show that microRNAs are an important agent in inducing of the bystander effect. [43].

In-Vivo studies have shown that when only one side of body is irradiated, chromosomal damages in the skin of mice increases. In these studies hypomethylation on skin was only observed on the area under direct radiation but the level of Dnmts in the shield area was decreased. Also cranial irradiation leads to the reduction of methylation in the long interspersed nuclear element 1 (LINE1) and expression reduction in DNA methyltransferase 3A of the spleen. Reduced expression of DNA methyltransferase 3A enzymes was attributed to the micro RNA-194 in this survey which is a significant factor in regulation of this enzyme expression. Further studies have confirmed the increase in expression of this microRNA even several months after irradiation of the spleen [43, 44].

Studies suggest that changes in methylation pattern after irradiation is tissue-dependent and methylation levels may vary in different tissues. Cranial irradiation causes lasting hypomethylation in the spleen while a short time hypomethylation may be observed in the skin [45]. Changes in miRNAs expression are significantly regarding to gender. Assessment of male and female mice spleen after cranial irradiation showed that some miRNAs in females and some other miRNAs in males had higher expressions. Also gonadectomy of mice leads to changes in miRNA expression in both males and females. These results indicate that sex hormones have role in miRNA expression

and chromosome methylations after irradiation [46].

### The bystander effect can cause epigenetic heritage

As it was noted, epigenetic changes are heritable and can be transmitted to future generations. Radiation can lead to genomic instability in generic cells and affect the oncoming generations. In-Vivo studies have reported that the bystander effects can affect the generic cells to change the methylation pattern in future generations. Cranial irradiation is associated with increase of chromosomal damage in mouse sperms. Furthermore, studying the level of methylation at CCGG sites shows a significant decrease in methylation of the testes and sperm. Evaluation of the thymus, spleen, liver and bone marrow in the next generation of mice which were irradiated by their cranial area depicted that level of methylation and enzymes of DNA methyltransferase is diminished in the bone marrow, thymus and spleen significantly, however, no reduction was observed in the liver. Most of these changes have been observed in the bone marrow respectively[47].

Further studies pointed that the expression of MIR-29a and MIR-29b in the germ cells and the next generations of mice which were irradiated by their cranial area were significantly high. These two expressions regulate the enzymes of DNA methyltransferase 3a and 3b. Therefore, increase of MIR-29a and MIR-29b reduces the production of DNA methyltransferase enzymes which ultimately diminishes the level of methylation.[48].

### Radioprotective effect of the bystander effect

Bystander effect may be a protective mechanism. Bystander effect may decrease the risk of transformation in the irradiated multicellular organism. The organism may be able to detect damage from irradiation and respond

to it by removing of damaged cells. It seems, apoptosis is the best way to remove of damaged cells. Apoptosis allows the elimination of damaged cells without a negative impact on other cells via inflammatory responses. The second way to remove injury is to quick damaged cells into permanent differentiation. Following exposure to low dose radiation, tissues remove all potentially damaged cells from the system to keep away from the risk of carcinogenesis. It seems, the bystander effect induced differentiation play vital role in this process. Obviously cellular senescence is a powerful tumor suppressor mechanism. Based on this theory, irradiated tissue would respond as a single unit. The damaged cells would produce some the bystander signals.

### Radiotherapy and the bystander effect

More than half of cancer patients use the radiotherapy for tumor treatment[49]. The risk of second cancers caused by radiation exposure maintain for long years after radiotherapy. Second cancer is one of complications of radiotherapy and it may lead to a decrease in the overall survival after the treatment of primary cancers. The bystander effect is one of the complications of radiation exposure which cause chromosomal damage and may lead to carcinogenesis risk in distant tissues. Studies have also shown that the bystander effect is more conspicuous in fractionated doses[50]. So the bystander effect could have an impact at the incidence of second cancers in the distant tissues in radiotherapy. Several major cancers that could have a link with these effects including cancers of the lung, bronchus, melanoma and sarcoma after radiotherapy for prostate cancer[8]. The high incidence of lung cancer in patients with rectal, cervical and ovarian cancer radiotherapy has been confirmed [51-53]. High incidence of these cancers cannot be due to scatter radiation. That is why it is thought that the bystander effect

plays a role in the development of these cancers. According to these results, the cancer risk due to bystander effects in patients with cancer which use of radiotherapy for treatment are be considered.

### Result and Conclusion

Radiation-induced bystander effect is an important biological response that leads to damages very similar to direct irradiation in distant non-irradiated cells and tissues. Nowadays several mechanisms which are involved in the bystander effects have been discovered. Chromosome damages induced by free radicals which cause bystander effect are not created by direct irradiation. These free radicals are produced by some cytokines, enzymes and genes expression. Mitochondria can increase production of free radicals in oxidative stress situation. Epigenetic modulators like methylation pattern and miRNAs are important changes which are investigated by the bystander effect. The methylation pattern and miRNAs which regulate gene expression could be involved in oxidative stress and radiation induced carcinogenesis.

### Conflict of Interest

None

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