

The Role of Epigenetic Modulation in the Cellular Response to Ionizing Radiation

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Received: March 13, 2015 Revised: April 6, 2015

Accepted: April 8, 2015

Published online: June 2, 2015

ABSTRACT

More and more evidence demonstrate that epigenetic modulation plays important roles in many cellular processes and carcinogenesis. It also showed that epigenetic changes are involved in the cellular response to ionizing radiation. In current review, we will discuss the radiation-induced epigenetic modifications including DNA methylation changes, chromatin remodelling and alterations in microRNA expression, and their roles in the cellular response to ionizing radiation. The aim is to help understand the mechanisms underlying the radiation induced biological effects in cells and to find the future research interests.

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Key words: Epigenetic modulation; DNA methylation; Chromatin remodelling; MicroRNA; Ionizing radiation

Pan D, Du YR, Hu BR. The Role of Epigenetic Modulation in the Cellular Response to Ionizing Radiation. *International Journal of Radiology* 2015; 2(1): 1-14 Available from: URL: <http://www.ghrnet.org/index.php/ijr/article/view/1109>

INTRODUCTION

Epigenetic alterations are heritable changes underlying gene expression by regulating the structure and function of the genome that are not caused by alterations in the DNA sequence itself. It includes an array of molecular modifications such as DNA methylation, chromatin remodelling, histone modifications, genetic imprinting, random chromosome(X) inactivation and noncoding RNA (microRNA, lincRNA and siRNA, etc) regulated gene expression^[1]. The principal mechanisms of epigenetic changes are alterations in DNA methylation and alterations to the packaging of DNA around the core histones, both of which can result in gene activation or repression^[2,3].

Epigenetics plays important roles in many cellular processes from gene expression to cellular proliferation and an aberrant epigenetic state in cells can result in carcinogenesis^[4]. Changes in genomic DNA methylation post-irradiation and its potential correlation with cellular response have been indicated^[5-7], and changes in expression profiles of microRNA (miRNA) have been observed following irradiation in mouse and human cells. These evidences suggests that epigenetics may be the core mechanistic link between irradiation and cellular response. Here, we mainly review the DNA methylation changes, chromatin remodelling and alterations in miRNA expression, and their roles in the cellular response to ionizing radiation (IR).

DNA METHYLATION

DNA methylation is one of the most important epigenetic modifications of the genome involving in the regulation of numerous cellular processes through gene silencing without altering DNA sequences. It is the most extensively studied epigenetic mechanism and refers to the addition of a methyl group (-CH₃) to the carbon 5 position of cytosine ring in a CpG dinucleotide by DNA methyltransferase (DNMTs)^[8,9]. The remaining CpG dinucleotides in the mammalian genome are often methylated. CpG dinucleotides are not uniformly distributed throughout the human genome. Rather, they are concentrated in specific regions (CpG islands) that are located in the upstream region from the transcriptional start site of many genes

as well as at other regions. In human, approximately 60% of all genes have CpG islands in their promoter regions, and these promoter associated CpG islands are generally unmethylated in normal cells, with the exception of genomic imprinting, X-chromosome inactivation or tissue specific gene hypermethylation^[10,11]. Cancer research in epigenetics in the 1990s was dominated by a focus on understanding and extending the discoveries of DNA methylation abnormalities^[4]. DNA methylation plays a central role in the epigenetic control of genomic programs in both normal and cancer cells^[12]. Of which the majority is unmethylated in all normal tissue types and throughout development, which was demonstrated by using computational analyses^[13].

In mammals, there are four types of DNMT: DNMT1, DNMT2, DNMT3a and DNMT3b. DNMT1 functions as the maintenance methyltransferase by copying the methylation pattern of the old DNA strand onto the newly synthesised strand during DNA replication^[14]. In cancer and embryonic stem cells unmethylated CpG sites are targeted for de novo methylation by DNMT3a and DNMT3b^[15]. Information about Dnmt2-dependent methylation patterns has been limited to a few isolated loci and the results have been discussed controversially. In mammals DNMT2 is not a DNA methyltransferase. It was shown that Dnmt2 has a robust methyltransferase activity toward cytosine 38 in the anticodon loop of tRNA-Asp and other tRNAs and that the enzyme uses the conserved DNA methyltransferase mechanism to methylate tRNA^[16]. In agreement with this notion, several independent phylogenetic analyses have suggested that Dnmt2 is an ancient DNA methyltransferase that has switched its substrate specificity from DNA to tRNA^[16-18]. The DNMT2 protein methylates C38 of tRNA-Asp and it has a role in cellular physiology and stress response and its expression levels are altered in cancer tissues^[19].

In cancer cells, the promoter-associated CpG islands of tumor suppressor genes (TSGs) may be aberrantly methylated (or hypermethylated), thereby promoting recruitment of methyl-binding domain proteins, and histone-modifying and chromatin-remodeling complexes to the methylated promoter-associated CpG sites^[20]. This renders formation of a compact chromatin configuration, precluding access of transcription factors and hence transcriptional silencing of the corresponding TSGs^[21]. Therefore, DNA methylation is an important mechanism leading to the inactivation of protein-coding or noncoding genes in human cancers. Promoter regions of TSGs are hypomethylated to allow their expression and maintain the normal state of the cell^[12]. Cells that have mutations in DNMT lack the stabilise effect of DNA methylation and have prominent nuclear abnormalities^[22].

DNA METHYLATION AND RADIATION

DNA methylation is one of the most common mechanisms of epigenetic regulation. Accumulating evidence suggests that changes in methylation patterns may help mediate the sensitivity or resistance of cancer cells to IR. The present studies provide evidence for the involvement of radioresistance-induced DNA methylation changes in tumor radioresistance. There are multiple reports of altered DNA methylation patterns following exposure to IR in plants, rodents, and rodent and human cell lines^[5,6,23-28]. Plants when exposed to IR showed dose-dependent hypermethylation^[29]. IR exposure has been found to have dose-dependent, sex, and tissue-specific effects on global hypomethylation using mouse model system^[30]. Mostly hypomethylation, loss of methylation, paralleled with a decrease in the DNA expression levels of methyltransferases (DNMTs; DNMT1, DNMT3a, and DNMT3b) and methyl CpG binding proteins (MeCP2)

was associated with radiation-induced changes^[25,29].

In many of these studies, the global DNA hypomethylation observed post-irradiation along with the decreased expression of proteins involved in maintaining the correct genomic methylation patterns suggest that these changes might play a role in cellular response^[6,25,29]. X-ray irradiation of mice was also shown to affect methylation of the promoter of the p16 tumour suppressor in a sex- and tissue-specific manner, but not the methylation status of O-6-methylguanine-DNA methyltransferase (MGMT)^[31]. It was suggested that the radiation-induced hypomethylation patterns might result in genomic instability and reshuffling of the hereditary material via chromosomal instability or reactivation of transposable elements, allowing the cells to survive in the radiation environment.

DNA damage and genomic instability were manifested as reproductive cell death, reduced cloning efficiency at successive passages, increased apoptosis, and an increased proportion of chromosome aberrant cells after irradiation, while the cellular responses to ionizing radiation are shown to be predominantly associated with DNA hypermethylation of certain CpG dinucleotides and pericentromeric spermidine N1-acetyltransferase 2 (SAT2) satellite sequences. Non-specific DNA hypomethylation is also observed. The majority of the hypermethylated sequences detected are repeat elements. Aberrant methylation status of repeat elements and SAT2 satellite sequences are known to affect cell survival and genomic instability^[32,33]. Based on these observations, the authors suggested that methylation alterations following radiation exposure are involved in.

Exposures to low linear energy transfer (LET) gamma-rays (γ -rays) were also shown to result in dose-dependent global hypomethylation in the C-1300 N1E-115 (mouse), CHO K-1 (hamster), V79A03 (hamster), and HeLa S-3 (human) cell lines^[23]. However, another study using one of the same cell lines, CHO K-1, showed no change in methylation after X-ray exposure^[5]. The different outcomes in these two studies might suggest differences in changes in epigenetic profiles after different types of radiations. The latter study also showed global hypomethylation in mouse liver tissue after X-rays exposure but demonstrated no change in mouse brain or spleen tissue^[5]. Even though each of these studies was performed post-low LET irradiation, different responses were observed.

There are interesting reports on the relationship between a DNA methyltransferase inhibitor (5-aza-2'-deoxycytidine) and radiation sensitivity in colon cancer. Genomic hypomethylation induced by 5-azacytidine results in enhanced radiation sensitivity in coloncancer^[34,35]. Promoter-CpG islands of five previously identified radioresistance-related genes (TOPO2A, PLXDC2, ETNK2, GF11, and IL12B) were significantly altered in the radioresistant laryngeal cancer cells. Furthermore, the demethylation of these gene promoters with a DNA methyltransferase inhibitor (5-aza-2'-deoxycytidine) increased their transcription levels. Treatment with 5-aza-2'-deoxycytidine also sensitized the radioresistant laryngeal cancer cells to irradiation, indicating that changes in DNA methylation contributed to their radioresistance^[36]. In addition, breast cancer cells treated with fractionated IR showed several locus-specific DNA methylation alterations, which were mostly loss of methylation (TRAPP9, FOXC1, and LINE1)^[37]. Together with the evidence for altered promoter methylation in particular cancers^[32,38], these data provide a link among radiation exposure, epigenetics and carcinogenesis. Radiation-induced global DNA hypomethylation and promoter hypermethylation may play roles in the maintenance of the genomic instability.

So far, studies have already indicated the global hypo- and

hypermethylation changes in the irradiated cells. However, it remains to know which genes in methylation changes are more important for cells after irradiation.

CHROMATIN REMODELLING

Chromatin remodelling is involved in many biological processes including gene expression, DNA replication and repair, chromosome condensation, segregation and apoptosis^[39]. Gene transcription is controlled by histone modifications in chromatin. Positioning of the nucleosome with its 147 base pairs of DNA wrapped around the octamer of the core histones, H2A, H2B, H3, and H4, in conjunction with the above modifications of histones, modulates the normal epigenome in terms of maintaining gene expression patterns and normal chromosome structure and function^[40].

Carcinogenesis not only alter DNA methylation but also cause global changes in the levels of proteins that participate in chromatin modifications, polycomb-complex components, and in histone modifications by acetylation and methylation of lysine residues on histones H3 and H4^[41,42]. The strong dependence between DNA methylation and chromatin modifications for DNA packaging is known. Furthermore, histone modification and DNA methylation closely interact in the setting of the transcriptional states of chromatin. Especially in cancer cells, silenced genes regulated by DNA hypermethylation can be models to examine the chromatin control of gene expression. When such genes are expressed with no methylation, their promoters have virtually identical distribution of the active marks, H3K9acetyl and H3K4me^[30,43]. In contrast, when silenced genes are associated with hypermethylation, the distribution of these active marks is severely decreased, and virtually every histone methylation mark, including mono-, di-, and trimethylation of H3K9 and H3K27 that has been associated with transcriptional repression, is enriched^[30].

Phosphorylation of histone H2AX at serine 139 (γ H2AX) is one of the most extensively studied histone modifications^[44]. γ H2AX is used as an early marker for induction and repair of DNA double-strand breaks^[45,46]. It also appears to play a functional role in the DNA repair process, maintenance of genome stability, and the early stages of cancer^[47,48].

Histone deacetylases (HDACs) catalyze the removal of acetyl groups on the histone tail, resulting in a transcriptionally inactive heterochromatic state^[11]. Likewise, SIN3A (part of an HDAC repressor complex) is downregulated in NSCLC^[49]. Relative to normal lung cells, lung cancer cells undergo H4K5/H4K8 hyperacetylation, H4K12/H4K16 hypoacetylation, and H4K20me3. Lower global levels of H4K20me3 can be detected in precursor lesions and is particularly common in squamous cancers^[50].

Epigenetic silencing of transcription also occurs through post-translational histone modification, chromatin remodelling and changes in the nuclear positioning of genes^[18]. Deregulation of these and other chromatin processes have been linked to the development and progression of cancer^[39,51,52]. Some chromatin remodelling modifications observed in cancer cells are alterations in the histone acetylation/deacetylation balance, increased or decreased poly-adenosine diphosphate (ADP)-ribosylation and failures in ATP-dependent chromatin remodelling mechanisms^[53]. Deregulation of histone acetylation can lead to carcinogenesis in three ways. Histone hypoacetylation at certain promoter regions, induced by decreased HAT activity or increased HDAC activity, can silence tumour suppressor genes. Conversely, histone hyperacetylation at other promoter regions, induced by elevated HAT activity or decreased

HDAC activity, can lead to the activation of oncogenes. Thirdly, carcinogenesis can be triggered by aberrant recruitment of HAT or HDAC^[11].

CHROMATIN REMODELLING AND RADIATION

IR induced DNA damage response generally occur in special euchromatic and heterochromatic regions. Chromatin immunoprecipitation (ChIP) and immunofluorescence analysis were used to explore the distribution of γ H2AX. ChIP experiments showed higher γ H2AX signal after IR in histone H3 trimethylated lysine 4 (H3K4me3), compared to lysine 9 (H3K9me3) enriched chromatin fragments, and this strengthen the dependence of IR-induced DNA damage response on the chromatin region^[54]. However, few studies try to investigate the radiation-induced histone modifications to indicate the epigenetic mechanism.

Radiation-induced phosphorylation of γ H2AX was extensively studied as a measure of DSB accumulation in irradiated cells^[55,56]. γ H2AX accumulates in the nucleus at DSBs forming the γ H2AX foci, and a direct correlation has been found between γ H2AX phosphorylation and the number of DSBs resulting from radiation. Therefore, γ H2AX is crucially important for the repair of DNA strand breaks and for the maintenance of genome stability^[44].

X-ray irradiation in a mouse model induced a decrease in trimethylation of histone H4K20 in the thymus and, eventually, resulted in an overall relaxation of the chromatin organization in cells. In addition, gamma-irradiation can result in relaxation of the chromatin structure around the DSB immediately after exposure. However, after some time, the methylation of H3K9 increased and the chromatin restored to the condensed state^[57]. The epigenetic mechanisms on histone modification after radiation exposure still remain to be explored.

In mice, radiation exposure led to decreased trimethylation of histone H4 lysine which might result in relaxed heterochromatin organisation and would impair genome stability^[26]. It has been shown in human cells that euchromatic regions were more susceptible to radiation-induced DNA damage and γ H2AX accumulation^[58-60]. Cells exposed to γ -rays showed chromatin decondensation at sites of double-strand breaks^[61]. The changes were manifested as a decrease in intensity of chromatin labeling, increased histone H4 lysine 5 acetylation, and decreased histone H3 lysine 9 dimethylation. Forty minutes post-irradiation, these changes induced by radiation exposure were replaced by histone modifications typical for condensed chromatin (decreased acetylation of histone H4 lysine 5 and increased methylation of histone H3 lysine 9). The data suggested a requirement to convert from less to more condensed chromatin after DNA repair. Another study observed rapid binding of SWI/SNF complexes to chromatin in regions of double-strand breaks via interaction with γ H2AX, suggesting that the repair mechanisms were facilitated by SWI/SNF complex promotion of H2AX phosphorylation^[29]. The role of ataxia telangiectasia mutated (ATM) in some of the chromatin changes in response to radiation and as a result its potential role in cellular response to ionizing radiation have been implicated^[63]. In response to double-strand break induction, Kruppel-associated box (KRAB) associated protein (KAP-1) is phosphorylated in an ATM-dependent manner^[64]. Phosphorylated KAP-1 leads to euchromatinisation. Therefore, these results suggested that chromatin relaxation is a fundamental pathway in the DNA damage response and that ATM and KAP-1 are its primary mediators. It was also suggested that ATM-dependent heterochromatin relaxation is specifically required for the repair of double-strand breaks located

within heterochromatin^[65]. Another study identified another signaling cascade that helps initiate the DNA damage response by altering chromatin^[66]. These studies indicate that exposure to radiation affects chromatin remodelling in association with DNA damage and DNA repair. Radiation-induced aberrations in chromatin remodelling can lead to DNA damage and impaired DNA repair. This compromised DNA repair and relaxed heterochromatin contribute to the initiation of genomic instability.

MICRORNA

MicroRNAs (miRNAs) belong to a class of single strand, small noncoding RNAs of 19-25 nucleotides (nts) in length that regulate diverse developmental and pathological processes in eukaryotic organisms^[67-69]. It can regulate negatively the expression of up to hundreds of messenger RNA (mRNA) targets and are dysregulated frequently in lung cancer. Depending on their genomic locations, miRNA genes can be transcribed from two different pathways: intergenic miRNAs are transcribed by RNA polymerase II as primary miRNAs (pri-miRNAs) with independent transcription units. The primary transcripts (pri-miRNAs) are generated by polymerase II and stabilized by 5' capping and 3' polyadenylation^[70]. The distinct hairpin secondary structure of pri-miRNA is recognized by the microprocessor complex (Drosha and DGCR8) and then specifically cleaved at the base of stem-loop releasing a 60-80 nts pre-miRNA. A pre-miRNA of the hairpin or stem-loop structure is exported into cytoplasm by exportin 5, and further processed and cleaved by Dicer to yield the mature miRNA. Mature miRNAs lead to translational repression or mRNA degradation of the target protein-coding genes by binding to complementary sequence within 3' untranslated region (3' UTR) of their target mRNAs^[71]. After completing its task, the mature miRNA is degraded by the 5'-3' exoribonuclease or 3'-5' exoribonucleases. In cancer cells, approximately 50% of annotated miRNA genes are mapped to fragile sites of the human genome, deletion of which is recurrently identified in cancer^[72]. Over the past decades, it has become clear that aberrantly expressed miRNAs contribute to the initiation or progression of human cancers^[73]. Moreover, emerging evidence demonstrated that miRNAs can function as oncogenes or tumor suppressors^[74].

MICRORNA EXPRESSION AND RADIATION

MiRNAs as critical gene regulators can influence signaling pathways that alter multiple cellular processes, including the DNA damage response (DDR) after IR. Increasing evidence demonstrates that the expression levels of miRNAs significantly change after IR, which suggests that miRNA play important roles in the IR-caused DDR^[75]. While changes in expression profiles of miRNA have been observed following irradiation in mouse and human cells^[76,77], the first evidence for functional importance of a miRNA in radiation responses was shown in *Drosophila*^[78]. Following irradiation of fly larvae, the bantam miRNA was shown to be up-regulated. This miRNA in turn was shown to bind to the 3'-UTR of the pro-apoptotic gene and affect its post-transcriptional down-regulation. This miRNA-mediated gene repression resulted in decreased apoptosis induced by radiation and increased larvae survival. In mouse embryonic stem cells, a group of miRNA and their putative gene targets that are potentially involved in response to DNA damage, were identified post-irradiation^[76].

In whole body irradiated mice, the spleen and thymus tissues showed sex-specific deregulation of miRNA expression. Among these, miRNA-34a (miR-34a) and miR-7 were thought to be involved

in counteracting radiation induced hypomethylation^[79]. MiR-709 target the Brother of the Regulator of Imprinted Sites (BORIS), an important regulator of DNA methylation and imprinting^[80]. This study showed that the radiation induced DNA damage resulted in increased miR-709 expression in exposed testes and decreased levels of BORIS prevent massive aberrant erasure of DNA methylation, resulted in downregulation of miRNA levels.

MiR-421 in neuroblastoma and HeLa cells downregulates ATM kinase, which is a crucial integrator of DNA DSBs repair machinery. Ectopic expression of miR-421 leads to S-phase cell cycle checkpoint changes and an increase in radiosensitivity^[81]. Recently, more miRNAs, including miR-18a, miR-100, miR-101, miR-181, have been identified as novel regulators to control the protein level of ATM^[82-85]. BRCA1, a critical tumor suppressor, is also recruited to DNA damage lesions, in which it facilitates DNA repair. The level of BRCA1 is regulated by miR-182, miR-146a, and 146b-5p and may impact breast cancer therapy and by deferring from homologous recombination^[86,87]. MiR-18a attenuates cellular repair of DNA double-strand breaks by directly suppressing ATM. Ectopic expression of miR-18a significantly inhibited the repair of DNA damage induced by etoposide, leading to accumulation of DNA damage, increase in cell apoptosis and poor clonogenic survival^[88]. Overexpression of miR-24, the first miRNA found to target H2AX, down-regulates the level of H2AX, resulting in higher sensitivity of cells to IR^[89]. Alternatively, the expression of miR-101 and miR-34a downregulated DNA-PKcs and p53 binding protein1, respectively, impeding the NHEJ repair pathway^[84,90]. The tumor suppressor p53 has a central role in the activation of genes in multiple pathways, including cell-cycle regulation, tumor suppression, and apoptosis. miR-125b and miR-504 have been identified as negative regulators of p53 in several types of human cells^[91,92]. Interestingly, miR-605, and miR-143/miR-145 are post-transcriptionally activated by p53 and, subsequently, target Mdm2, leading to rapid accumulation of p53^[93,94]. Radiation treatment downregulate the levels of miR-521 and upregulate DNA repair protein Cockayne syndrome protein A (CSA). Similarly, ectopic inhibition of miR-521 results in increased CSA protein levels and plays an important role in the radiosensitivity of prostate cancer cell lines^[95]. Other miRNAs, such as miR-34, miR-21, have been shown to regulate the expression of important DDR network proteins BCL2, manganese superoxide dismutase (MnSOD), and MSH2, respectively^[96,97]. Following gamma-irradiation of human lung carcinoma A549 and human B lymphoblastic cells IM9, a number of miRNA showed more than 2-fold changes in irradiated cells^[98,99]. The target genes were involved in apoptosis, regulation of cell cycle, and DNA damage and repair. A study involving gamma-irradiation of human B lymphoblast cell lines showed dose-dependent changes in miRNA expression^[100].

Low dose X-rays irradiated cells (0.05 Gy) demonstrated a decrease in miR-20 and miR-21^[101]. MiR-20 and miR-21 also increase in tumorigenesis and miR-21 is considered as an onco-miRNA. In high dose irradiated cells (10 Gy), miR-197 is upregulated. Carcinogenic characteristics are observed in normal cells following injection of miR-197, suggesting that stimulation of expression of miR197 by high dose radiation can lead to the progression of tumorigenesis. In addition, ionizing radiation-induced oxidative stress has been shown to play a role in altering miRNA expression^[101]. The role of miRNA in the immune system and in inflammation has been reviewed in the literature^[102,103]. The mechanism of epigenetic regulation in vivo is thought to be linked to inflammatory processes in the radiation response. Therefore, these miRNA play important roles in the initiation and/or perpetuation^[104].

In vitro three-dimensional (3D) growth of tumors is a cell culture model that more closely mimics the features of the *in vivo* environment and is being used increasingly in the field of biological and medical research^[105]. It has been demonstrated that cancer cells cultured in 3D matrices are more radio-resistant compared to cells in monolayers^[106]. However, the mechanisms causing this difference remain unclear. Our experimental results showed that more miRNAs were down regulated in three dimensional (3D) cultured human lung epithelial cells (HBED-3KT), compared to the two dimensional (2D) cultured HBEC-3KT cells after X-ray irradiation and carbon-beam irradiation as well. The significantly different expression of miRNAs such as miR-1202, miR-1290, miR-205 may be crucial in inhibiting the expression of target proteins and, therefore, lead to the different cellular response in 2D and 3D cultured cells^[107].

Together, these studies suggest that radiation exposure alters miRNA profiles in a way that can affect oxidative stress, DNA damage, DNA repair, regulation of cell cycle, apoptosis, tumorigenesis and changes in DNA methylation. MiRNA serve as integrators of the cellular response to ROS and DNA strand breaks, both of them are results of ionizing radiation. Further investigation of miRNA impact on cellular sensitivity to DNA-damaging agents will be in favor of cancer therapy.

CONCLUSION AND PERSPECTIVE

Based on above mentioned, we think that radiation exposure can induce epigenetic changes including alterations in DNA methylation, chromatin remodelling and miRNA expression. Epigenetic changes are linked to alterations in global, repeat element and tumour suppressor promoter methylation. Radiation exposure can induce relaxed heterochromatin organisation. At low doses radiation, miRNA changes are involved in suppressing radiation-induced apoptosis, stimulating DNA repair, counteracting radiation-induced damage, and suppressing the progression of cancer but higher doses of radiation lead to tumorigenic progression. However, some mechanisms underlying the radiation-induced cellular response such as genomic instability still remains largely unknown. The evidence presented in this review supports that epigenetic mechanisms may be involved in. Induction of epigenetic changes may be an initiating factor in the instability. These changes are heritable and persist in the progeny of the irradiated cells. Additionally or alternatively, epigenetic alterations may be a late arising delayed effect of irradiation driving the perpetuation of the cellular response. Epigenetic regulation has recently been established as an emerging tool of cancer therapy. Therefore, the understanding of the epigenetic mechanism in cancer and radiation-induced cellular response is required for the development of cancer radiotherapy. It is also imperative to investigate more precise mechanisms involving in specific gene or protein in order to better control carcinogenesis or prevent human from radiation risk.

ACKNOWLEDGEMENTS

The work was supported by the National Nature Science Foundation of China to BH [31170803, U1432121], and the National Key Scientific Instrument and Equipment Development Project of China to BH [2012YQ03014210].

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