

Review Article

Epigenetic Changes and Nontargeted Radiation Effects—Is There a Link?

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It is now well accepted that the effects of ionizing radiation (IR) exposure can be noticed far beyond the borders of the directly irradiated tissue. IR can affect neighboring cells in the proximity, giving rise to a bystander effect. IR effects can also span several generations and influence the progeny of exposed parents, leading to transgenerational effects. Bystander and transgenerational IR effects are linked to the phenomenon of the IR-induced genome instability that manifests itself as chromosome aberrations, gene mutations, late cell death, and aneuploidy. While the occurrence of the above-mentioned phenomena is well documented, the exact mechanisms that lead to their development

have still to be delineated. Evidence suggests that the IR-induced genome instability, bystander, and transgenerational effects may be epigenetically mediated. The epigenetic changes encompass DNA methylation, histone modification, and RNA-associated silencing. Recent studies demonstrated that IR exposure alters epigenetic parameters in the directly exposed tissues and in the distant bystander tissues. Transgenerational radiation effects were also proposed to be of an epigenetic nature. We will discuss the role of the epigenetic mechanisms in radiation responses, bystander effects, and transgenerational effects. *Environ. Mol. Mutagen.* 49:16–25, 2008. © 2008 Wiley-Liss, Inc.

Key words: radiation; indirect effects; bystander effect; transgenerational genome instability; epigenetics

DIRECT EFFECTS OF RADIATION EXPOSURE

All living organisms are exposed to ionizing radiation (IR) on a day-to-day basis. In addition to diagnostic and therapeutic medical radiation exposures, most radiation exposures today are chronic, with primary sources being background radiation, cosmic rays, radioactive waste, radon decay, nuclear tests, and accidents at the Chernobyl and other nuclear power plants. On the one hand, IR is a well-known cancer-inducing agent. The carcinogenic potential of IR was recognized very soon after its discovery, when the first radiation-induced tumor was reported in 1902 [Little, 2000; 2003]. Over the past decade, intense research efforts have been made to elucidate the cellular and molecular mechanisms of radiation-induced carcinogenesis in eukaryotic and, most importantly, mammalian cells. On the other hand, radiation is one of the primary clinical methods used for detecting and fighting human malignancies, which makes the understanding of the underlying mechanisms of IR effects much more imperative.

IR can affect a variety of processes in exposed cells. It can cause changes in gene expression, disruption of mitochondrial processes, cell cycle arrest, and apoptotic cell death [Amundson and Fornace, 2003; Belyakov et al., 2002, 2003, 2006; Criswell et al., 2003; Fei and El-Deiry,

2003; Iliakis et al., 2003; Powell and Kachnic, 2003; Andreev et al., 2006; Jeggo and Lohrich, 2006; Rodemann and Blaese, 2007; Valerie et al., 2007]. The most important is that IR is a potent DNA damaging agent capable of producing DNA damage such as cross linking, nucleotide base damage, and single and double strand breaks [Little, 2000]. The accumulation of DNA damage caused by IR in conjunction with disrupted cellular regulation processes can lead to carcinogenesis [Little, 2000; Barcellos-Hoff, 2005; Sawant et al., 2001; Sowa et al., 2006].

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INDIRECT RADIATION EFFECTS: RADIATION-INDUCED GENOME INSTABILITY AND BYSTANDER EFFECTS

Historically, the central dogma of radiation biology stated that the effects of IR were restricted to the directly hit cells. This paradigm has been challenged by numerous observations in which cells that were not directly traversed by the IR exhibited responses similar to those of the directly irradiated cells. These responses were demonstrated in the cells that were descendants of the directly irradiated cells and were termed radiation-induced genome instability [Morgan, 2003a,b,c; Morgan and Sowa, 2005, 2007]. Genomic instability is characterized by an increased rate of acquisition of alterations in the genome. It manifests itself as an induction of chromosomal aberrations, aneuploidy, micronuclei, gene mutations and amplifications, microsatellite instability, and cell death [Morgan, 2003a,b; Suzuki et al., 2003]. Radiation induced genomic instability displays in the irradiated cell at delayed times after irradiation and in the progeny of the irradiated cell generations after exposure [Little, 2000]. There are many signaling pathways involved in the initiation and perpetuation of genomic instability [Kaplan et al., 1997]. The relative contribution of the different pathways depends upon the genetic background of the irradiated cell or organism [Paquette and Little, 1994; Watson et al., 1997]. It has long been proposed that genomic instability may play a significant role in tumorigenesis. Currently, genome instability is thought to be a hallmark of many cancers if not an important prerequisite for cancer formation [Goldberg, 2003; Little, 2003].

Radiation responses were also seen in the naïve “bystander” cells that were in contact with irradiated cells or received signals from the directly irradiated cells. These bystander effects also challenge the classic radiation biology dogma [Morgan, 2003a,b; Mothersill and Seymour, 2003, 2004, 2006; Morgan and Sowa, 2005]. The radiation induced bystander effects encompass a number of different endpoints. Some, but not all, of these are detrimental to the cell. Similar to genomic instability, bystander effects are measured by the induction of gross genome rearrangements, chromosome aberrations, sister chromatid exchanges, deletions, duplications, mutations and amplifications, and cell death [Lorimore et al., 2001; Zhou et al., 2002; Suzuki et al., 2003; Klovov et al., 2004; Lorimore et al., 2005; Smilenov et al., 2006; Hamada et al., 2007; Han et al., 2007]. Bystander effects have been observed following cytoplasmic irradiation, demonstrating that the target for genetic events is not only the nucleus [Randers-Pehrson et al., 2001]. They were also seen in cells that were not traversed by radiation, but were in the same environment as the irradiated cell. These bystanders received signals from the irradiated cells that generated a response in the bystander cells. Evidence suggests that the bystander effects are communicated

between the cells by means of either the gap junction intercellular communication or by the transmission of soluble factors between the irradiated cell and the nonirradiated cell through the cell culture medium. Among the candidate soluble factors are reactive oxygen species and cytokines [Morgan, 2003a,b,c].

Responses seen in nonirradiated bystander cells include cell death, neoplastic transformation, and genomic instability [Mothersill et al., 2001; Zhou et al., 2002, 2005; Azzam and Little, 2004; Azzam et al., 2004; Suzuki and Tsuruoka, 2004; Suzuki et al., 2004; Maguire et al., 2005; Yang et al., 2005, 2007; Lyng et al., 2006; Hu et al., 2006; Liu et al., 2006; Gaugler et al., 2007; Han et al., 2007; Maguire et al., 2007]. Bystander effects have also been observed in three-dimensional tissue models including spheroids [Persaud et al., 2005], and in the reconstructed human tissue models [Belyakov et al., 2005; Sedelnikova et al., 2007]. As a result, bystander effects are accepted as an ubiquitous consequence of radiation exposure [Mothersill and Seymour, 2004].

Bystander effects also manifest themselves in the whole-organism context. Yet, compared to the bystander effect data based on the cell culture, the conclusive data on the somatic bystander effects in vivo are relatively scarce [Goldberg and Lehnert, 2002; Hall, 2003; Koturbash et al., 2006, 2007; Mothersill et al., 2007]. It was shown that radiation exposure led to the release of soluble “clastogenic” factors into the circulating blood. These factors are capable of inducing chromosome damage in the cultured cells. Such clastogenic activity was found in plasma of the patients receiving a high dose radiotherapy and in individuals accidentally exposed to radiation [Goh and Summer, 1968; Pant and Kamada, 1977; Emerit et al., 1994, 1995; Marozik et al., 2007]. Bystander effects were also shown to be important within an exposed organ. When the lung base was irradiated, the significant molecular and cellular damage was observed in the shielded lung apex [Khan et al., 1998, 2003]. The same group also showed that exposure of one lung, either right or left, resulted in a marked increase of micronuclei in the unexposed, shielded lung [Khan et al., 1998, 2003]. Similar within-the-organ bystander effects were observed during partial liver irradiation [Brooks, 2004; Brooks et al., 1974]. Recently, the existence of the somatic bystander effects was confirmed using the rodent skin and spleen models, whereby one part of the animal body was exposed to IR, while the other part was protected by a medical grade shield [Koturbash et al., 2006, 2007]. The data showed that X-ray exposure to one side of the animal body caused profound changes in the unexposed bystander portion of the body.

While a great deal of data has been accumulated on the existence and manifestation of genomic instability and bystander effects in cultured cells, 3D tissues, organs and organisms, the mechanisms of these enigmatic phenomena

remain unexplored. High frequency of induction and persistence of the bystander responses suggests their possible epigenetic background [Lorimore et al., 2003; Morgan, 2003a,b; Nagar et al., 2003; Kaup et al., 2006; Wright and Coates, 2006].

TRANSGENERATION RADIATION-INDUCED EFFECTS

The effects of radiation exposure can also span several generations in animals or humans. Evidence to support the hypothesis for heritable effects from the parental germline radiation exposures in humans has been the subject of much debate. However, the data from animal models have clearly demonstrated that effects of the parental radiation exposure are transmitted through the germline to the progeny of the irradiated parent [Morgan, 2003a,b,c]. Early studies of these transgeneration effects used various tests such as the specific locus, dominant lethal, and heritable translocation assays [Generoso et al., 1980; Russell and Kelly, 1982; Green et al., 1987; Russell et al., 1998]. Other studies focused on heritable alterations in cancer incidence and teratogenesis following the parental preconception irradiation [Mohr et al., 1999; Pils et al., 1999; Nomura, 2003; Nomura et al., 2004; Dasenbrock et al., 2005]. In addition to this classic evidence for transmitted effects, it is now apparent that risks to the progeny of irradiated parents also include transgeneration genomic instability [Carls and Schiestl, 1999; Mohr et al., 1999; Barber et al., 2002, 2006; Shiraishi et al., 2002; Dubrova, 2003a,b; Morgan, 2003b; Niwa, 2003; Nomura, 2003; Nomura et al., 2004; Slovinska et al., 2004; Dasenbrock et al., 2005; Tawn, 2005; Barber and Dubrova, 2006; Singer et al., 2006].

While the exact molecular mechanisms of transgeneration radiation-induced genome instability have yet to be discovered, some evidence points to an epigenetic nature of these phenomena [Morgan, 2003b; Jirtle and Skinner, 2007]. Specifically, several independent studies have shown that the IR-induced cellular reprogramming persists for multiple generations. Wiley et al. used a mouse preimplantation embryo chimera assay to demonstrate heritable effects of paternal irradiation on embryonic cell proliferation that persisted for two generations with no decrease in the incidence or severity of the effect between generations [Wiley et al., 1997]. These observations led them to hypothesize that a non-Mendelian mode of inheritance was involved. Later Vance and Wiley [1999] showed that by blocking gap junction intercellular communication they could “rescue” the irradiated embryo, allowing normal cell proliferation in the irradiated embryonic component of the embryo chimera. These data demonstrated that the decreased cell proliferation rate observed in the embryo from the irradiated parent occurred as a result of gap junction mediated signaling between the irradiated

embryo and the control embryo. They also demonstrated that offspring from F₀ parental irradiation exhibited pronounced biochemical alterations [Baulch et al., 2001; Vance et al., 2002, Baulch and Raabe, 2005]. Four generations of offspring from the irradiated sires exhibited changes in protein kinase C, mitogen-activated protein kinase, Tpr53 and p21^{waf1} levels. These changes were not consistent in magnitude or direction of change within the offspring of a given generation, nor were they consistent between generations [Baulch and Raabe, 2005]. The authors again hypothesized that the expression of these genes might be altered through an epigenetic process.

Further, high frequency persistent heritable genetic effects on embryonic and spermatogenic cell proliferation rates that spanned over 2–3 generations of animals following paternal irradiation also suggested an epigenetic pattern of regulation of these transgeneration effects [Wiley et al., 1997; Baulch et al., 2002].

The possible mechanism that may underlie the observed persistent transgenerational gene expression phenotype was termed “epigenomic instability” [Baulch and Raabe, 2005]. Recent studies using the neutral pH sperm comet assay have also demonstrated the effects on sperm DNA electrophoretic mobility in sperm of irradiated male mice 7-weeks postirradiation [Baulch et al., 2007; Li and Baulch, 2007]. This same assay also demonstrated an unconventional, heritable DNA damage effect, or changes in chromatin conformation, in nonirradiated offspring of irradiated male mice [Baulch et al., 2007; Li and Baulch, 2007]. These findings further support the proposed “epigenomic instability” theory.

Dubrova et al. pioneered the analysis of the transgeneration instability of repeat sequences in the genome [Dubrova et al., 1993]. Initially, these repeat sequences were termed minisatellites, but later they were renamed the expanded simple tandem repeat (ESTR) loci. This change in terminology emphasized that ESTR loci are less stable short repeats (4–6 bp), while true minisatellites tend to be more stable and generally consist of longer repeats (6–100 bp) [Dubrova, 2003b]. The ESTR analysis was used to demonstrate the induction of heritable genomic instability in the progeny of irradiated mice [Dubrova et al., 1993; Barber et al., 2002; Yauk et al., 2002]. The analysis of repeat elements in the genome has not only been applied to the transgeneration mouse model, but has also been utilized to study transgeneration effects in human populations including the atomic bomb survivors, individuals affected by the Chernobyl accident, the Chernobyl clean up workers, and those living around the Semipalatinsk nuclear test site [Dubrova et al., 1996, 1997, 2002].

As with other studies of transgeneration effects, the high magnitude non-Mendelian inheritance of increased ESTR mutation rates suggested the epigenetic deregulation as a possible causative factor [Barber et al., 2006].

EPIGENETIC CHANGES AND THEIR ROLES IN THE CELL

Epigenetic changes are meiotically heritable and mitotically stable alterations in gene expression that include DNA methylation, histone modification, and RNA-associated silencing [Jaenisch and Bird, 2003]. Cytosine DNA methylation was the first epigenetic alteration identified, and is the most widely studied epigenetic mechanism. It is crucially important for normal development, cell proliferation, and proper maintenance of genome stability of a given organism [Jaenisch and Bird, 2003; Baylin, 2005; Baylin and Ohm, 2006; Jirtle and Skinner, 2007]. In mammals DNA methylation occurs predominantly in the context of CG dinucleotides that are methylated to 60–80% [Weber and Schuebeler, 2007].

DNA methylation is known to be associated with inactive chromatin state and in most cases with repressed gene expression activity [Hendrich and Tweedie, 2003; Klose and Bird, 2006; Weber and Schuebeler, 2007]. Altered global DNA methylation patterns are a well-known characteristic of cancer cells [Baylin, 2005; Baylin and Ohm, 2006; Weidman et al., 2007]. The global loss of DNA methylation has been linked to the activation of transposable elements, elevated chromosome breakage, aneuploidy, increased mutation rates and, thus to the phenomenon of global genomic instability [Robertson, 2002; Weber and Schuebeler, 2007; Weidman et al., 2007].

Undoubtedly, changes in DNA methylation are not isolated events, and they occur in the context of the global chromatin deregulation and altered histone modification levels [Jaenisch and Bird, 2003; Weidman et al., 2007]. Histone modifications including acetylation, methylation, phosphorylation, and ubiquitination are important in the transcriptional regulation [Jenuwein and Allis, 2001; Weidman et al., 2007]. Histone acetylation is linked to transcriptional activation, while histone deacetylation generally represses transcription [Jenuwein and Allis, 2001]. Histone methylation can result in different transcriptional consequences depending upon the residue affected. Furthermore, histones can be mono-, di-, and tri-methylated. This adds a vast complexity to the yet unexplored histone code [Cheung and Lau, 2005; Saha et al., 2006; He et al., 2007; Weidman et al., 2007].

Phosphorylation is another important histone modification [He et al., 2007]. One of the best-studied modifications is phosphorylation of histone H2AX. H2AX becomes phosphorylated at serine 139 (γ H2AX), possibly as one of the earliest cellular responses to double strand breaks (DSBs). It is crucially important for DSB repair and for the maintenance of genome stability [Rogakou et al., 1998; Pilch et al., 2003; Sedelnikova et al., 2003].

Finally, epigenetic control can also be mediated by small regulatory RNAs [Bernstein and Allis, 2005]. Among them, microRNAs (miRNAs) are of a special interest. MicroRNAs are abundant, small, single-stranded noncod-

ing RNAs that regulate gene expression. These molecules are conserved across species [Hwang and Mendell, 2006; Sevignani et al., 2006]. To control the translation of the target mRNAs, miRNAs associate with the RNA-induced silencing complex (RISC) proteins and bind to the 3'UTR of mRNAs, thus serving as translational suppressors that regulate the protein synthesis [Hutvagner and Zamore, 2002]. Regulatory miRNAs impact cellular differentiation, proliferation, apoptosis and, possibly, even predisposition to cancer [Chang and Mendell, 2007; Fabbri et al., 2007]. Aberrant levels of miRNAs have been reported in a variety of human cancers [Volinia et al., 2006; Wiemer, 2007]. Furthermore, it was recently suggested that small RNAs may be involved in the regulation of chromatin packaging [Grewal and Moazed, 2003; Bernstein and Allis, 2005].

EPIGENETIC CHANGES IN THE DIRECTLY EXPOSED TISSUE

Direct radiation exposure strongly influences epigenetic effectors. DNA damaging agents including IR have been reported to affect DNA methylation patterns [Kalinich et al., 1989; Tawa et al., 1998; Minamoto et al., 1999; Kovalchuk et al., 2004]. Acute exposures to low LET X-rays or γ -rays were noted to result in global hypomethylation [Kalinich et al., 1989; Tawa et al., 1998]. It was recently shown that the IR exposure leads to the profound dose-dependent and sex- and tissue specific global DNA hypomethylation [Pogribny et al., 2004, 2005; Raiche et al., 2004; Koturbash et al., 2005; Loree et al., 2006]. The IR exposure also affects methylation of the promoter of the p16 tumor suppressor in a sex- and tissue-specific manner [Kovalchuk et al., 2004]. The DNA hypomethylation observed after irradiation was related to DNA repair [Pogribny et al., 2004]. It also correlated with the radiation-induced alterations in the expression of DNA methyltransferases, especially de novo methyltransferases DNMT3a and DNMT3b [Raiche et al., 2004; Pogribny et al., 2005]. Most importantly, the radiation-induced global genome DNA hypomethylation appeared to be linked to genome instability in the exposed tissue [Pogribny et al., 2004, 2005; Raiche et al., 2004; Loree et al., 2006].

DNA methylation is closely connected to other components of chromatin structure. Although much attention has been given to the radiation-induced changes in DNA methylation, histones have been largely overlooked. Among the histone modifications that change upon radiation exposure, phosphorylation of histone H2AX is being studied most intensively. Histone H2AX, a variant of histone H2A, is rapidly phosphorylated at Ser139 upon the induction of DNA strand breaks by irradiation, and it can be effectively visualized within repair foci using phospho-specific antibodies [Sedelnikova et al., 2003]. Recent studies have also indicated that radiation-induced global loss of DNA methylation may correlate with the changes

in histone methylation, specifically with the loss of histone H4 lysine trimethylation [Pogribny et al., 2005]. The data on the IR effects of microRNAome are in their infancy [Ishii and Saito, 2006; Marsit et al., 2006].

EPIGENETIC DETERMINANTS OF THE INDIRECT RADIATION EFFECTS: BYSTANDER EFFECT

Even though a significant body of evidence points toward the epigenetic nature of the radiation-induced bystander and transgenerational effects, until recently very few studies addressed the exact epigenetic changes related to the indirect radiation response. The pioneering work of Kaup et al. has shown that DNA methylation is important for the maintenance of the radiation-induced bystander effect in cultured cells. Using cultured human keratinocytes, they demonstrated that the dysregulation of DNA methylation in naïve cells exposed to the medium from the irradiated cells persists for 20 passages. Over a similar period of culture under the similar conditions, these cells have also exhibited increased and persistent levels of chromosome and chromatid aberrations, reproductive cell death, apoptosis, and other signs of genome instability [Kaup et al., 2006].

A wide range of extensive cell-culture based studies address the role of phosphorylated histone H2AX in bystander effects [Sokolov et al., 2005; Smilenov et al., 2006; Burdak-Rothkamm et al., 2007; Yang et al., 2007].

Epigenetic changes were also shown to be important in whole-tissue- and whole-organism-based bystander effect models. The reconstituted 3D human tissue model offers an excellent alternative to cell cultures. The recent study by Sedelnikova et al. examined bystander effects in two reconstructed human 3D tissue models, airway and full-thickness skin. Following the microbeam irradiation of cells located in a thin plane through the tissue, a variety of biological endpoints were analyzed in distal bystander cells (up to 2.5 mm away from the irradiated cell plane) as a function of postexposure time (0 hr–7 days). They detected a significant increase in the levels of phosphorylated H2AX in bystander tissues and extensive long-term increases in apoptosis and micronucleus formation, as well as the loss of nuclear DNA methylation, persistent growth arrest, and the increasing number of senescent cells. Of a special interest is the observed loss of DNA methylation in bystander cells. DNA methylation is an important epigenetic phenomenon involved in the regulation of gene expression and genome stability. Since changes in DNA methylation are linked to other epigenetic effectors, the observed alteration of DNA methylation in bystander cells may be indicative of an epigenetic nature of bystander effect in 3D human tissue models [Sedelnikova et al., 2007].

Further insight into the role of epigenetic changes in bystander effects comes from the animal-based studies, where irradiation was shown to induce DNA damage and

modulate the epigenetic effectors in distant bystander tissues. The Kovalchuk and Engelward laboratories pioneered in studies on the role of epigenetic changes in radiation-induced bystander effects in vivo. To analyze in vivo bystander effects, they developed a mouse model whereby half of an animal body was exposed to radiation, while the other half was protected by a medical grade shield [Koturbash et al., 2006]. This model was used to monitor the induction and repair of DNA strand breaks in the cutaneous tissue. In addition to this well-established endpoint, the authors also explored the possibility of epigenetic mechanisms (i.e. DNA methylation and alterations in DNA methyltransferases and methyl-binding proteins) in the generation and/or maintenance of a radiation-induced bystander effect in cutaneous tissue. They have shown that radiation exposure to one half of the body leads to elevated levels of DNA strand breaks, and altered levels of key proteins that modulate methylation patterns and silencing in the bystander half of the body at least 0.7 cm from the irradiated tissue. These are some of the first data to clearly demonstrate that the epigenetically regulated bystander effects occur in vivo in distant tissues. Importantly, these epigenetic changes in bystander tissues are not due to the insufficient shielding or radiation scattering [Koturbash et al., 2006].

To be relevant for carcinogenesis, the epigenetic manifestations of bystander effects should accumulate and/or persist over a long period of time. To investigate the possibility that the localized X-ray irradiation induces persistent epigenetically modulated bystander effects in distant tissues, Koturbash et al. monitored the occurrence of epigenetic changes (i.e. DNA methylation, histone methylation and miRNA expression) in spleen tissue 7 months after the localized cranial irradiation. This analysis has revealed that the localized cranial radiation exposure leads to the decreased levels of global DNA methylation. It also alters the levels of key proteins that modulate methylation patterns and silencing (i.e. de novo methyltransferase DNMT3a and methyl-binding protein MeCP2) and contributes to the reactivation of the LINE1 retrotransposon in the bystander spleen, located at least 16 cm from the irradiation site. Importantly, it is the first evidence that the down regulation of DNMT3a and MeCP2 is probably triggered and maintained by higher activity of a small regulatory RNA, micro RNA *miR-194*. These experiments have demonstrated that *miR-194* is up-regulated in the bystander rat spleen. These data have also clearly demonstrated that the bystander effect occurs in vivo in distant tissue, persists over a long period of time, and is epigenetically regulated [Koturbash et al., 2007].

The observed altered expression of *miR-194* in the bystander rat spleen was very intriguing and promoted further studies of microRNAome changes in bystander tissues. Using the microRNA microarray platform, microRNAome patterns have been profiled in skin and

spleen tissues of mice subjected to sham treatment, whole-body or head exposure. The radiation exposure led to very significant alterations in the microRNA expression profiles in bystander skin and spleen [Kovalchuk laboratory, unpublished observations]. The pronounced microRNAome alterations can also be seen in the bystander tissues using the 3D model [Sedelnikova and Kovalchuk, unpublished observations]. These preliminary data suggest that the microRNA expression changes really occur in bystander tissues. Their exact function in the bystander effect has still to be delineated. Furthermore, due to their small size and high stability, microRNAs may be plausible candidates for the bystander signal.

EPIGENETIC DETERMINANTS OF THE INDIRECT RADIATION EFFECTS: TRANSGENERATION EFFECTS

To date, a wealth of evidence has been accumulated on the nature of transgenerational changes in the somatic tissues of the progeny of exposed parents [Barber and Dubrova, 2006]. Notwithstanding, the exact molecular mechanisms leading to the radiation-induced transgenerational genome instability and carcinogenesis remain elusive. The occurrence of genome instability and elevated mutation rates in the progeny of exposed parents was attributed to some, yet unknown, mechanisms, possibly epigenetic mechanisms [Jirtle and Skinner, 2007]. Relatively few studies have addressed the potential epigenetic alterations in offspring of irradiated parents.

The first direct evidence of the epigenetic effectors involvement in transgenerational responses comes from the recent study by Koturbash et al. They utilized an *in vivo* mouse model to analyze the role of epigenetic parameters in the transgeneration radiation effects [Koturbash et al., 2006].

In this study, the C57Bl/6 mice were exposed to 2.5 Gy of X rays and mated 7 days after exposure. Several mating groups were established: maternal exposure only, paternal exposure only, and both parents exposure. Mock-treated mating pairs served as controls.

To test whether changes in DNA methylation were observed in the somatic tissue of offspring, global DNA methylation was measured in spleen, thymus, and liver of offspring. A significant loss of DNA methylation was observed in the thymus of offspring upon paternal and combined parental exposure. The DNA methylation changes were correlated with the alterations in the levels of DNA methyltransferases and methyl-binding proteins. Specifically, DNMT1 expression was dramatically decreased in the thymus tissue of the progeny of exposed males and the progeny with the combined paternal and maternal exposure. The levels of DNMT3a and 3b were also significantly down-regulated in the progeny of exposed males and in the combined parental exposure

group. The decrease in global cytosine DNA methylation and DNMTs levels observed in the thymus of the progeny upon paternal and combined parental irradiation were correlated with a significant decrease in the level of methyl-binding protein MeCP2. This protein selectively recognizes methylated DNA and is important for methylation-mediated gene silencing and chromatin remodeling. It has also been implicated in carcinogenesis [Yu et al., 2001; Jaenisch and Bird, 2003; Koturbash et al., 2006]. Mammalian genomes heavily depend upon properly set patterns of methylated cytosines for their function. The global loss of DNA methylation and altered levels of DNMT1, DNMT3a or 3b can lead to the activation of transposable elements contributing to genome instability [Xu et al., 1999; Yu et al., 2001; Jirtle and Skinner, 2007]. Therefore, it can be suggested that the global loss of DNA methylation observed in the progeny of irradiated parents may influence retrotransposons and satellite DNA, thus underlying transgenerational genome instability. Importantly, such a speculation may help explain the satellite DNA instability in the progeny of exposed parents [Barber and Dubrova, 2006]. The most recent unpublished data from the Kovalchuk laboratory suggest that the transgeneration DNA methylation alterations affect satellite DNA, tubulin loci, and a variety of short interspersed nuclear elements.

Parental irradiation has also resulted in a significant elevation of levels of phosphorylated histone H2AX in the thymus tissue of progeny of exposed males and both exposed parents. The observed accumulation of phosphorylated H2AX is an important epigenetic alteration, and it may be an early sign of predisposition to carcinogenesis [Sedelnikova and Bonner, 2006].

Another important indicator of the involvement of epigenetic effectors-histone modifications (phosphorylated histone H2AX) in the transgeneration radiation-induced changes comes from the study by Barber et al. They analyzed the transgeneration mutation rates and DNA damage in the germline and somatic tissues of the first generation offspring of the irradiated inbred mice of two different strains. In both strains, the strongly elevated transgeneration mutation rates have been exhibited. The authors attribute the elevated mutation rates to persistently elevated levels of phosphorylated histone H2AX. This finding underscored once again the importance of phosphorylated H2AX in the indirect radiation effects and radiation-induced genome instability [Barber et al., 2006]. Furthermore, this study suggests that the persistence of elevated mutation rates in the tissues of the offspring may be due to the epigenetic inheritance of instability signals through sperm. DNA methylation is proposed to be a plausible candidate for an epigenetic signal that leads to transgenerational mutagenesis. Altered DNA methylation levels in sperm may profoundly affect the fertilized embryo [Aitken and De Iuliis, 2007]. Methylation can be transmitted over many cell divisions. It can result in the long-

term gene expression changes by affecting genes responsible for the genome stability maintenance. The recent study by Hatch et al. has once again reinforced the conclusion that DNA methylation plays an important role in the transgenerational changes in mutation and recombination rates in the progeny of exposed animals [Hatch et al., 2007]. Notwithstanding the exact mechanisms of transgenerational DNA methylation, the inherited phenotypic and genotypic changes need to be further analyzed. Specifically, the role of unrepaired DNA damage, altered DNA methylation, and chromatin in the sperm cells needs to be unraveled [Aitken and De Iuliis, 2007].

The possible function of small regulatory RNAs in the transgenerational radiation effects is the least explored and has also to be further investigated. Small RNAs may constitute another epigenetic signal that can be passed on through the sperm cells, thus influencing chromatin packaging and gene expression in the fertilized egg.

CONCLUSIONS AND OUTLOOK

Epigenetic parameters seem to be the plausible mediators of the indirect radiation effects, including the radiation-induced genome instability, bystander, and transgenerational effects. DNA methylation and histone modification changes directly impact chromatin packaging, and therefore influence the gene expression and susceptibility of DNA to rearrangements. Short RNAs due to their small size and relative stability may be acting as signals or mediators of the indirect radiation effects. Further studies including those that use transgenic and knockout models of epigenetic mediator genes may shed more light on the interrelationship between the genetic rearrangements and epigenetic effects.

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