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Biochemical and Biophysical Research Communications 325 (2004) 39–47
BBRC
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Sex- and tissue-specific expression of maintenance and de novo DNA methyltransferases upon low dose X-irradiation in mice

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Received 23 September 2004

Abstract

DNA methylation is crucial for normal development, proliferation, and proper maintenance of genome stability for a given organism. A variety of DNA damaging agents that are known to affect genome stability were also shown to alter DNA methylation patterns. We have recently pioneered the studies in the area of the radiation effects on DNA methylation, and found that radiation exposure led to substantial dose-dependent and tissue-specific DNA hypomethylation, which was much more pronounced in spleen and liver of female animals. The exact mechanisms of radiation-induced DNA hypomethylation are still to be uncovered. We have previously shown that one of those mechanisms may potentially be DNA repair related. Another possible mechanism may be linked to changes in the expression of DNA methyltransferases (DNMTs). In the current study, we examined the radiation-induced changes in expression of maintenance DNMT1, and de novo methyltransferases DNMT3a and DNMT3b in spleen and liver of irradiated animals. This was paralleled by the studies of acute and chronic IR-induced methylation changes in spleen and liver of intact animals, as well as in animals with altered sex hormone status. Here we report that radiation-induced DNA methylation changes correlated with radiation-induced alterations in expression of DNA methyltransferases. We present the data on tissue-specificity in radiation-induced expression of DNA methyltransferases, and prove that changes in the expression of de novo methyltransferases DNMT3a and DNMT3b are the most important in radiation-induced DNA methylation alterations. We also discuss the role of sex hormones, especially estrogen, in the generation of the sex-specific radiation-induced methylation changes.

Keywords: Radiation; DNA methylation; DNA methyltransferases; Sex hormones

Mammalian cells epigenetically modify their genomes by means of DNA methylation—a phenomenon crucially important for normal development, proliferation, and proper maintenance of genome stability for a given organism [1–5]. DNA methylation in mammalian cells largely occurs on cytosine residues at CpG dinucleotides in genomic DNA.

Cytosine methylation of CpG sites is widely observed in vertebrate DNA, and various lines of evidence support its involvement in allele-specific gene inactivation [3,4]. DNA methylation is tissue- and species-specific [6–8], and DNA methylation status is known to change throughout senescence [9]. As well, aberrant cytosine methylation is quite common in cancer development [10]. Concordantly, methylation changes in cancer cells include global genome hypomethylation combined with de novo methylation and transcriptional repression of certain promoter regions in cancer cells [10–12]. Most importantly, changes in global DNA methylation patterns have been linked to the phenomenon of genomic instability and increased mutation rates [13,14].

A variety of DNA damaging agents that are known to affect genome stability were also shown to alter DNA methylation patterns [15]. Ionizing radiation (IR)—a potent mutagen and carcinogen—is one of

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doi:10.1016/j.bbrc.2004.10.002

those agents and its effects have been widely characterized and studied [7,8,15,16].

Studies of IR-induced DNA methylation changes are at their dawn. Kalinich et al. [17] reported that exposure of cell lines to γ rays led to the reduction of DNA methylation levels. In 1998, Tawa et al. [16] have described the induction of genome-wide hypomethylation upon in vivo and in vitro radiation exposure. Both studies however were conducted using in vitro cell line approaches and single radiation doses, but environmentally and clinically relevant exposures, occurring predominantly under chronic/repetitive low-dose whole-body exposure conditions, were never examined.

We have recently pioneered these studies in the area of low-dose radiation effects and compared the effects of acute and chronic whole body irradiation on DNA methylation in various tissues using in vivo murine model [7,8]. Our results suggested that epigenetic alterations in murine cells following irradiation were dose-dependent, and sex- and tissue-specific [7,8].

The most noticeable changes were observed in spleen and liver, where single dose IR exposure led to strong, significant, and dose-dependent DNA hypomethylation. Notably, radiation-induced DNA hypomethylation was much more pronounced in female animals.

These striking observations have piqued our attention and promoted us to further investigate possible mechanisms of the observed sex differences. Thus, we have decided to study the role of sex hormones in generation and/or maintenance of radiation-induced epigenetic changes.

The exact mechanisms of radiation-induced DNA hypomethylation are poorly understood. We have previously shown that one of the mechanisms of radiation-induced DNA hypomethylation may potentially be DNA repair related [8]. This information aside, we could not exclude the fact that other mechanisms, such as changes in the expression and/or activity of methylation maintaining enzymes, may also be involved in radiation-induced DNA hypomethylation. DNA methyltransferases 1 (DNMT1), DNMT3a, and DNMT3b are three functional methyltransferases known to contribute to methylation patterning in mammalian cells [18–22]. Disruption of their activity may result in improper methylation patterns. Notably, no data exist on the effects of IR on methyltransferase activity/expression in mammalian cells. We therefore examined the radiation-induced changes in expression of DNA methyltransferases DNMT1, DNMT3a, and DNMT3b in spleen and liver of irradiated animals. This was paralleled by the studies of acute and chronic IR-induced methylation changes in spleen and liver of intact animals as well as in animals with altered sex hormone status.

In this study we used low, environmentally relevant, doses of IR applied acutely or in a chronic/repetitive mode. Here we report that chronic low dose IR exposure led to a significant loss of global genome DNA methylation in female mice, which was paralleled by a significant decrease in expression of DNA methyltransferases. We also discuss the role of sex hormones, especially estrogen, in the generation of the sex-specific radiation-induced methylation changes.

Materials and methods

Irradiation of animals

In the course of the study 24 C57/Bl6 mice (12 males and 12 females, Charles River, ON, Canada) were divided into three groups: control group, acutely exposed group, and group subjected to chronic/repetitive exposure for 10 days. All animals were 45 days old at the start of the experiment, had comparable body weights, and were housed in a virus-free facility. All the animals involved in this study were maintained according to the guidelines set out by the Canadian Council on Animal Care (Canadian Council on Animal Care, 1993). Mice from the 'chronic/repetitive' group were exposed to whole body irradiation of 50 cGy applied as 5 cGy of X-rays per day (0.2 cGy/s) for 10 days to mimic chronic repetitive exposure. The 'acute' group of mice was irradiated with 50 cGy (0.2cGy/s) of X-rays once, on the 10th day of treatment of the 'chronic' group. To insure consistency in animals handling, control and acute groups animals were mock-treated every day. All animals were sacrificed 3 h after the last treatment on day 10. Spleen and liver tissues (left, median, and one caudate lobes) were sampled immediately upon sacrifice and divided into two equal parts, frozen, and used for DNA and protein extraction.

The experiment was replicated using 18 C57/Bl6 mice (9 males and 9 females). Both experiments yielded congruent data.

Effect of sex hormones on radiation-induced DNA methylation responses

Two independent experiments were conducted. During the course of each experiment 18 female C57/Bl6 mice were ovariectomized (OVX cohort) under general isoflurane anaesthesia. Ten days after the operation 9 animals were subjected to 17-β estradiol (estrogen) supplementation (OVX+ cohort) using a mini-pellet (0.05 mg/pellet, 21 day release) implantation (Innovative Research of America, Sarasota, FL, USA). These pellets are designed to release a total of 0.05 mg estradiol at a continuous rate of over 21 days. The OVX and OVX+ cohorts were divided into control, acute, and chronic/repetitive exposure groups and irradiated in accordance with the protocol described above. All animals were 45 days old at the start of irradiations. Both experiments yielded congruent data.

DNA methylation analysis

DNA extraction. Total DNA was prepared from liver and spleen tissues using Qiagen DNeasy kit according to the manufacturer's protocol (Qiagen, Qiagen, Mississauga, ON, Canada).

Cytosine extension assay to detect sequence specific changes in DNA methylation. DNA was digested overnight with a 10-fold excess of the methylation-sensitive restriction endonuclease, *Hpa*II, according to the manufacturer's protocol (New England Biolabs, Beverly, MA, USA). An additional DNA aliquot was incubated without restriction enzyme addition to serve as a background control. The single nucleotide extension reaction was performed in a 25 μL of DNA, 1× PCR buffer II, 1.0 mM $MgCl_2$, 0.25 U *Taq* DNA polymerase (Fisher Scientific, Nepean, Ontario, Canada), and [^{3}H]dCTP (57.4 Ci/mmol) (Perkin–Elmer, Boston, MA, USA) mix, incubated at 56 °C for 1 h, and then placed on ice. Duplicate aliquots from each reaction were placed on

Whatman DE-81 ion-exchange filters and washed three times for 10 min with sodium phosphate buffer (500 mM, pH 7.0) at room temperature. The filters were dried and processed by liquid scintillation counting (Beckman Instruments, Fullerton, CA, USA). Background label incorporation was subtracted from enzyme-digested samples and results were expressed as relative [^{3}H]dCTP incorporation/1μg of DNA or as percent change from control [7,8,23].

Western immunoblotting

Western immunoblotting for DNMT1, DNMT3a, and DNMT3b was conducted using spleen and liver tissues. Tissue samples were sonicated in 0.4–0.8 ml of hot 1% sodium dodecyl sulphate (SDS) and boiled for 10 min. Small aliquots (10 μl) of homogenate were reserved for protein determination using protein assay reagents from Bio-Rad (Hercules, CA, USA). Normalized aliquots of proteins (20 μg) were separated by SDS–polyacrylamide electrophoresis (PAGE) in slab gels of 12% polyacrylamide, made in duplicate, and transferred to PVDF membranes. Membranes were incubated with antibodies against DNMT1 (1:1000; Abcam, Cambridge, MA, USA), DNMT3a, and DNMT3b (both 1:500, Abgent, San Diego, CA, USA). Antibody binding was revealed by incubation with horseradish peroxidase-conjugated secondary antibodies (Amersham, Little Chalfont, UK) and the ECL Plus immunoblotting detection system (Amersham, Little Chalfont, UK). Chemiluminescence was detected by Biomax MR films (Kodak, Rochester, NY, USA). Unaltered PVDF membranes were stained with Coomassie Blue (Bio-Rad, Hercules, CA, USA) and the intensity of the M_r 50,000 protein band was assessed as a loading control. Signals were quantified using ImageJ 1.32j Software and normalized to both GAPDH and the M_r 50,000 protein, which gave internally consistent results (values relative to M_r 50,000 are plotted).

Statistical analysis

The main statistical procedures were described by Sokal and Rohlf [24]. Statistical treatment and plotting of the results were performed using Sigma Plot and Excel for Windows XP software. The results were presented as mean values ± standard error.

Results

Effect of sex hormones on acute and chronic LDR-induced global methylation changes in spleen and liver

In the course of the current study we assessed global genome methylation in spleen and liver tissues of male and female mice exposed to acute and chronic low-dose (0.5 Gy) whole body irradiation. We applied a well-established cytosine extension assay based on the use of the methylation-sensitive restriction enzyme *Hpa*II. This enzyme has relatively frequent recognition sequences at CpG sites throughout the genome, and leaves a 5′ guanine overhang following DNA cleavage that is ideal for subsequent single nucleotide extension with labeled [^{3}H]dCTP. The extent of [^{3}H]dCTP incorporation opposite the exposed guanine is directly proportional to the number of cleaved events, and by consequence, unmethylated CpG sites, but inversely proportional to the levels of methylation. Thus, the higher the methylation the lower the incorporation of [^{3}H]dCTP [7,8,23].

Initially, we evaluated the effects of LDR on DNA methylation in intact male and female animals and noted profound sex differences in the radiation-induced DNA methylation patterning of spleen and liver tissue. Female spleen chronic LDR exposure resulted in significant loss of DNA methylation ($p < 0.05$) (Fig. 1A). By

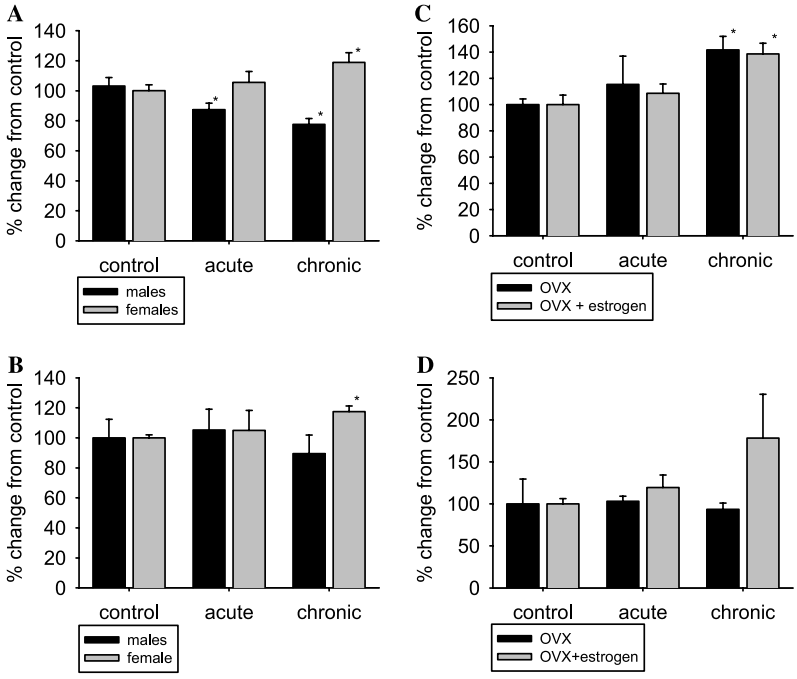

Fig. 1. Low dose radiation-induced DNA methylation changes. Levels of global genome DNA methylation in spleen and liver of irradiated mice measured by the cytosine extension assay. The results are presented as mean values ± SEM, $n = 4$; *$p < 0.05$. (A) Male and female spleen, (B) male and female liver, (C) ovariectomized and supplemented animal spleen, and (D) ovariectomized and supplemented animal liver.

contrast, both acute and chronic/repetitive exposure males displayed significant ($p < 0.05$) hypermethylation of spleen global genomic DNA (Fig. 1A).

Liver exposure (0.5 Gy of X-rays) led to significant decrease in DNA methylation only in chronically exposed females, while no changes were observed in males or in acutely exposed females. (Fig. 1B).

We hypothesized that the observed sex differences may be due to the possible role of sex hormones in the generation of a radiation epigenetic response. Estrogen (17-β estradiol) seemed to be the most likely 'affector,' based on the existing evidence for its role in carcinogenesis and its importance in setting of the methylation patterns in the germ cells [25].

To evaluate the role of sex hormones, in this case estrogen, in radiation-induced DNA methylation changes, we ovariectomized a cohort of female mice. Ten days after OVX operation half of the cohort was subjected to estrogen supplementation (OVX+ cohort) using continuous slow-release estrogen mini-pellets. The OVX and OVX+ cohorts were divided into control, acute, and chronic/repetitive exposure groups and exposed to 0.5 Gy of X-rays.

Notably, the spleen ovariectomy and estrogen supplementation did not result in changes of the 'intact female-like' DNA methylation response to LDR exposure (Fig. 1C).

Liver tissue, however, of the OVX animals exhibited a 'male-like' response (Fig. 1D). Significant DNA hypomethylation was not observed in the exposed OVX animals. Interestingly, estrogen supplementation led to a restoration of a 'female-like' chronic LDR-induced hypomethylation response in liver tissue (Fig. 1D).

Role of DNA methyltransferases in radiation-induced alterations of DNA methylation patterns

DNA (cytosine-5) methyltransferases 1 (DNMT1), DNMT3a, and DNMT3b are three main functional methyltransferases that are responsible for the establishing and maintaining of DNA methylation pattern in mammalian cells [19,21,22]. We hypothesized that radiation-induced deregulation of their expression may result in changes in DNA methylation.

We evaluated the effects of acute and chronic/repetitive LDR exposure on expression of DNMT1, DNMT3a, and DNMT3b in spleen and liver of male and female mice.

In the spleen, we found a strong and significant correlation between global genomic DNA methylation and expression of de novo DNA methyltransferases DNMT3a and DNMT3b ($r^2 > 0.9$ in all cases). Indeed, expression of DNMT3a and DNMT3b was significantly down regulated in chronically exposed female spleen tissue, paralleled by significantly decreased global DNA methylation ($p < 0.05$ in both cases) (Fig. 2).

In male spleen, where significant hypermethylation was observed following chronic exposure, we noted a strong and significant increase in the expression of DNMT3a and DNMT3b ($p < 0.05$) (Fig. 2).

No radiation-induced changes were found in expression of DNMT1 in either male or female spleen (Fig. 2).

Pronounced alterations in the expression of methyltransferases were also found in the liver tissue of exposed animals. In liver we observed a marked and significant ($p < 0.05$) decrease in the expression of

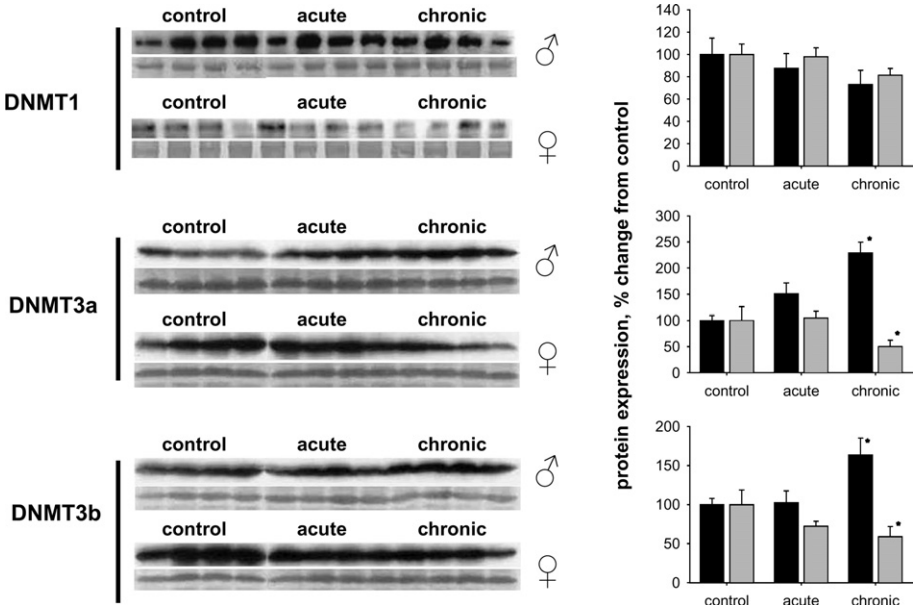

Fig. 2. LDR induced changes in expression of DNA (cytosine-5) methyltransferases in spleen of intact males and females. Lysates from spleen tissue were subjected to immunoblotting using monoclonal antibodies against DNMT1, DNMT3a, and DNMT3b. Protein levels relative to those of control animals are shown as the mean ± SEM; *$p < 0.05$, Student's t test. Black bars, males; grey bars, females. Sample loading was normalized to protein content. Representative Western blots from among two independent experiments are shown.

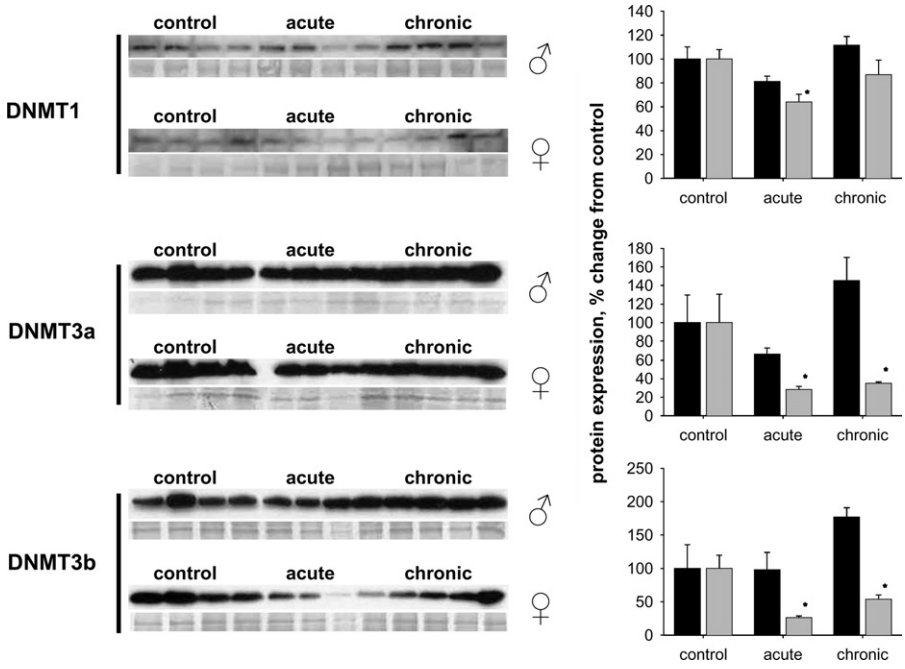

Fig. 3. LDR induced changes in expression of DNA (cytosine-5) methyltransferases in liver of intact males and females. Lysates from liver tissue were subjected to immunoblotting using monoclonal antibodies against DNMT1, DNMT3a, and DNMT3b. Protein levels relative to those of control animals are shown as the mean ± SEM; $*p < 0.05$, Student's t test. Black bars, males; grey bars, females. Sample loading was normalized to protein content. Representative Western blots from among two independent experiments are shown.

DNMT3a and DNMT3b in the acutely exposed female group and of all three DNMTs in the chronically exposed group. On the other hand, in male liver, LDR exposure did not affect the expression of any DNA methyltransferase (Fig. 3).

Effect of sex hormones on radiation-induced changes in DNA methyltransferase expression

Next we pursued the analysis of radiation-induced changes in methyltransferase expression in ovariecto-

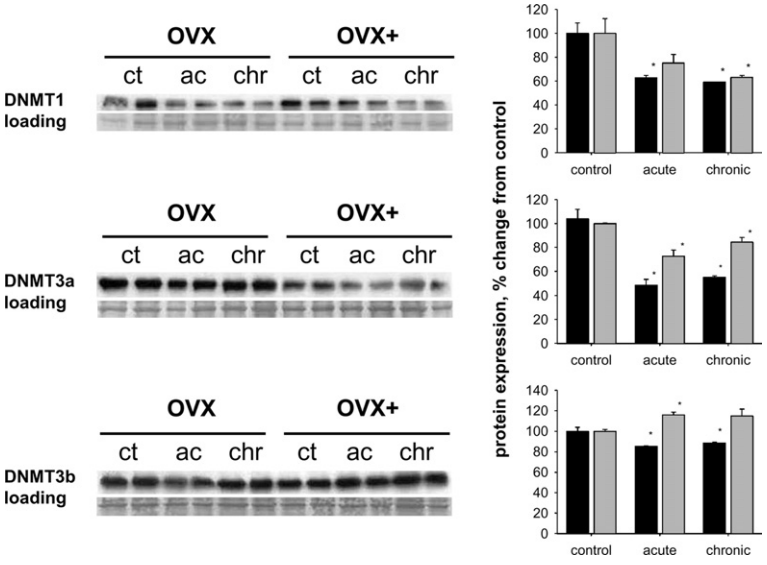

Fig. 4. Effect of sex hormones on DNMT1, DNMT3a, and DNMT3b expression in spleen. Lysates from spleen tissue of ovariectomized animals (OVX) and animals supplemented with estrogen (OVX+ estrogen) were subjected to immunoblotting using antibodies against the listed proteins. Protein levels relative to those of control animals are shown as the mean ± SEM; $*p < 0.05$, Student's t test. Black bars, OVX groups; grey bars, OVX+ estrogen groups. Sample loading was normalized to protein content. Representative Western blots from among two independent experiments are shown.

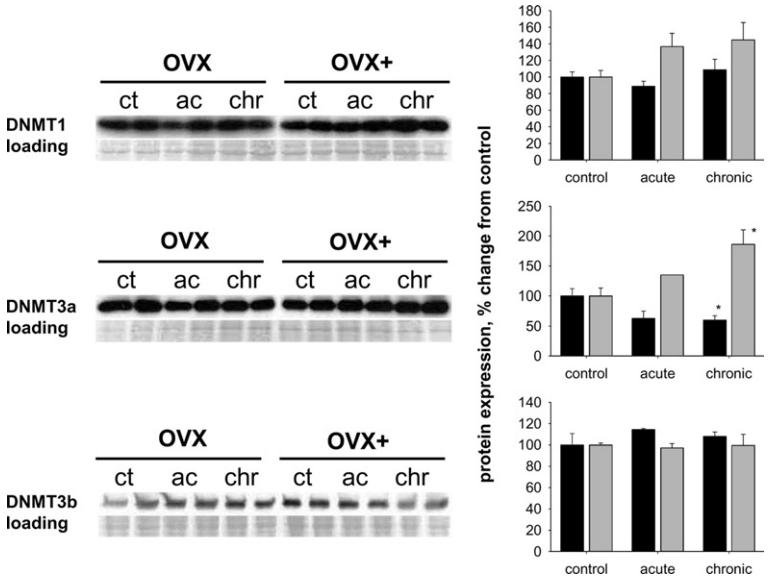

Fig. 5. Effect of sex hormones on DNMT1, DNMT3a, and DNMT3b expression in liver. Lysates from liver tissue of ovariectomized animals (OVX) and animals supplemented with estrogen (OVX+ estrogen) were subjected to immunoblotting using antibodies against the listed proteins. Protein levels relative to those of control animals are shown as the mean ± SEM; $*p < 0.05$, Student's t test. Black bars, OVX groups; grey bars, OVX+ estrogen groups. Sample loading was normalized to protein content. Representative Western blots from among two independent experiments are shown.

mized mice and in mice subjected to estrogen supplementation upon ovariectomy. Ovariectomy resulted in changes of radiation-induced methyltransferase expression patterns in spleen and liver tissues (Figs. 4 and 5). In spleen tissue, where no significant radiation-induced changes of DNMT1 expression were previously observed in the intact animals, we noted a significant decrease of DNMT1 expression in both OVX animals or animals subjected to estrogen supplementation (Fig. 4).

By contrast, expression of DNMT3a and DNMT3b was significantly down regulated by acute and chronic LDR exposure in spleens of ovariectomized mice ($p < 0.05$ in all cases), resembling intact female-like patterns in the chronic group. Surprisingly, estrogen supplementation of the OVX animals led to an increase in the expression levels of DNMT3b upon acute and chronic exposure, in which case only the acute group was significant. Expression of DNMT3a was still significantly down regulated by acute and chronic/repetitive LDR in estrogen-supplemented OVX animals ($p < 0.05$). It should be noted though that the relative expression levels in the estrogen-supplemented cohort were slightly higher than in the ovariectomized cohort (Fig. 4).

Significant changes in the expression of DNMT1 and DNMT3b were not observed in liver of either ovariectomized or estrogen supplemented mice upon LDR exposure. Similar to spleen, expression of DNMT3a in liver of OVX mice was down regulated by chronic LDR exposure, resembling the down-regulation of the intact animals (Fig. 5). Estrogen supplementation resulted in an increase of radiation-induced DNMT3a expression upon chronic exposure (Fig. 5).

Discussion

Ionizing radiation is a well-documented potent mutagen and carcinogen that induces a plethora of deleterious genetic as well as epigenetic effects [26–30]. DNA methylation is one of the crucial epigenetic mechanisms implicated in human carcinogenesis and in maintenance of the genome stability [10,12–14]. We reported previously that ionizing radiation exposure profoundly perturbed global genome DNA methylation in vivo in a sex- and tissue-specific manner [7,8]. The present study was designed to explore the possible mechanisms of the sex differences in radiation-induced DNA methylation changes of various tissues.

We hypothesized that sex hormones may contribute to the generation of sex-specific radiation-induced DNA methylation responses. We used the highly sensitive and *Hpa*II cytosine extension method to evaluate the acute- and chronic low dose whole body irradiation-induced DNA methylation changes of intact male and female animals, and in animals with altered sex hormone status (ovariectomized with or without estrogen supplementation). Based on the results of previous studies, we have chosen to work with the spleen and liver tissues, where sex differences in radiation-induced methylation changes were the most pronounced [8].

This study confirmed the finding that radiation-induced changes are indeed sex- and tissue-specific. We observed a significant DNA hypomethylation upon chronic low dose exposure in female spleen tissue. By contrast, male spleen irradiation resulted in a strong hypermethylation of the genomic DNA. Interestingly

enough, our previous results have shown that chronic high dose exposure does not result in the changes of DNA methylation patterns [8]. The apparent discrepancy between the effects of chronic low- and high-dose irradiation deserves further attention in the future, as it may hold the key to understanding the non-linearity in radiation dose–responses. Chronic low dose exposure is known to lead to substantial genome destabilization. LDR-induced methylation changes may contribute to such a destabilization.

The present study confirmed our previously reported claims that there exists a profound tissue specificity in regard to radiation epigenetic responses [8]. Overall, the radiation-induced changes in liver tissue were less pronounced than in spleen tissue (Table 1).

The most important and novel outcome of this study was the correlation between the radiation-induced changes in expression of DNA methyltransferases and the global genomic DNA methylation levels (Table 1).

The strong and significant DNA demethylation in female spleen upon chronic LDR exposure was paralleled by a strong down-regulation of the expression of de novo (DNMT3a and DNMT3b) methyltransferases (Table 1, Figs. 1 and 2). In chronically exposed male spleen an increase in the expression of DNMT3a and DNMT3b led to a significant hypermethylation of the genome (Figs. 1 and 2, Table 1). In chronically exposed intact female liver DNA hypomethylation resulted from a decrease in the expression of both maintenance and de novo methyltransferases (Table 1, Figs. 1 and 3). In contrast, expression of DNMT1 was not changed in spleen of exposed animals upon irradiation. Interestingly, previous studies based on experiments with 5-aza-2′-deoxycytidine indicated that DNMT1 is not able to maintain the methylation of DNA by itself once the level of 5-methylcytosine had been reduced below a certain level [20].

We also observed a paradoxical increase of DNA methylation levels in acutely exposed male spleen, which occurred in the presence of minor and insignificant increase in DNMT expression. Thus, we found that de novo methyltransferases played an important role in methylation patterning upon irradiation of spleen tissue. Contrarily, DNMT1 expression in spleen of exposed hormonally intact animals was unaffected.

The pronounced role of de novo methyltransferases in methylation patterning in radiation-exposed tissues was previously never reported and therefore constitutes an important finding of this study. De novo DNA methyltransferases (DNMT3a and DNMT3b) were recently shown to play critical roles in gene silencing and methylation patterning [31,32]. Recent results showed that DNMT3a and 3b are distributed in nucleoplasm and are not associated with nuclear DNA replication sites during S-phase [33]. These enzymes, most probably, contribute to DNA repair synthesis-related DNA methylation patterning, previously reported by our group [8]. The exact relationship between radiation-induced repair DNA synthesis and the action of methyltransfrases still has yet to be established.

The precise mechanism of radiation-induced changes in the expression of de novo methyltransferases remains unknown. For this reason, the role of DNMT1 deserves special attention in the future, as it was previously shown to stimulate DNA methylation activity of DNMT3a and DNMT3b [34].

Our results also show that the balance of sex hormones is important for mounting of sex- and tissue-specific radiation-induced DNA methylation responses. Hormonal balance seems to play a crucial role in liver tissue. Indeed, significant global genome DNA methylation changes were not noted in ovariectomized females, when compared to the intact animals (Fig. 1, Table 1). Radiation, as well, did not induce any changes in methyltransferase expression in ovariectomized animals (Table 1, Fig. 5). Estrogen supplementation did not reverse this effect, suggesting that a balance between various sex hormones and/or certain concentrations of sex hormones may be required for mounting the female-specific radiation response.

Our data suggest that radiation-induced DNMT1 expression changes were dependent upon the hormonal status of the animals. Both in liver and spleen samples, an ovariectomy led to changes in the radiation responsiveness of DNMT1 expression (Table 1). The effect was more dramatic in spleen, where ovariectomy led to a significant decrease in radiation-induced expression of DNMT1. This interesting observation clearly deserves further attention in the future. DNMT1 is the

Table 1
Radiation-induced changes in global genome DNA methylation and expression of DNA methyltransferases in spleen and liver of C57 Bl/6 mice

	Spleen								Liver							
	♂		♀		OVX		OVX+		♂		♀		OVX		OVX+	
	ac	chr	ac	chr	ac	chr	ac	chr	ac	chr	ac	chr	ac	chr	ac	chr
Methylation	↑	↑	—	↓	—	↓	—	↓	—	—	—	↓	—	—	—	—
DNMT1	—	—	—	—	↓	↓	↓	↓	—	—	—	↓	—	—	—	—
DNMT3a	—	↑	—	↓	↓	↓	↓	↓	—	—	↓	↓	—	↓	—	↑
DNMT3b	—	↑	—	↓	↓	↓	↑	—	—	—	↓	↓	—	—	—	—

Ac, acutely exposed animals; chr, chronically exposed animals; OVX, ovariectomized group; OVX+, ovariectomized group supplemented with estrogen.

major methyltransferase in somatic tissues that has preference for hemimethylated DNA and is indispensable for maintenance of methylation patterns during DNA replication [22]. DNMT1 is also known to ensure the clonal propagation of tissue-specific methylation patterns [23,35]. Deregulation of DNMT1 expression may thus modulate methylation patterning. Preventing radiation-induced decreases in DNMT1 may protect the cells from DNA hypomethylation and genome destabilization.

Collectively, our data show that sex-specific radiation-induced DNA methylation changes depend upon changes in expression of DNA methyltransferases that also are tissue-specific. Previous research from Kalinich et al. [17] also observed radiation-induced DNA hypomethylation in the exposed cell lines, suggesting that the redistribution of DNA methyltransferase from the nucleus to the cytoplasm might be the cause. The aforementioned study, however, did not take into account any possible sex differences. On the other hand, demethylation caused by reduction of levels of DNA methyltransferases is believed to take place through replication either during cell division or during repair DNA synthesis [6,12,16].

Sex-specific DNA demethylation could also be attributed, at least in part, to the action of 5-methylcytosine DNA glycosylase (5-MCDG), an enigmatic enzyme that was deemed to possess active DNA demethylation activity [36,37]. An estradiol receptor was recently shown to potentiate, in vitro, the 5-MCDG activity. The line of evidence prompted us to hypothesize that 5-MCDG could also be playing a role in sex-specific radiation-induced DNA demethylation. We evaluated the expression of 5-MCDG following irradiation of intact and ovariectomized animals, and found that 5-MCDG was not affected by LDR (data not shown). This suggests that the enzyme is not involved in radiation-induced tissue- or sex-specific demethylation.

At this point it is not possible to exclude that the activation of some DNA demethylation enzymes, leading to active replacement of 5-methylcytosine with cytosine, may be involved in tissue- and sex-specific radiation-induced DNA demethylation [8,38].

In summary, this is the first report on the roles of maintenance and de novo DNA methyltransferases in radiation-induced sex-specific DNA methylation changes. We have evaluated the role of estrogen, and concluded that it may be involved, at least in part, in a tissue- and sex-specific modulation of radiation epigenetic responses. Furthermore, the first evidence of the causative role of DNA methylation changes in tumorigenesis in mice was recently proven [10]. In the light of this important discovery our findings may constitute an important step towards understanding of radiation-induced epigenetic changes, and, possibly, their relationship to radiation carcinogenesis.

Acknowledgments

We thank Sandie Stevenson and Igor Kovalchuk for critical reading of the manuscript, and Greg Silasi and Margaret Forgie for technical assistance. We are grateful to Dennis Fitzpatrick (Vice-President (Research)) and Christopher Nicol (Dean of Art's and Science) of the University of Lethbridge for financial support. Also supported by NSERC Operating grant to O.K. J.R. was supported by the AHFMR Undergraduate Research Scholarship.

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