Int. J. Radiation Oncology Biol. Phys., Vol. 66, No. 2, pp. 327–330, 2006

0360-3016/06/$–see front matter

ELSEVIER

doi:10.1016/j.ijrobp.2006.06.012

RAPID COMMUNICATION

EPIGENETIC DYSREGULATION UNDERLIES RADIATION-INDUCED TRANSGENERATIONAL GENOME INSTABILITY *IN VIVO*

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Purpose: Although modern cancer radiation therapy has led to increased patient survival rates, the risk of radiation treatment-related complications is becoming a growing problem. Among various complications, radiation also poses a threat to the progeny of exposed parents. It causes transgenerational genome instability that is linked to transgenerational carcinogenesis. Although the occurrence of transgenerational genome instability, which manifests as elevated delayed and nontargeted mutation, has been well documented, the mechanisms by which it arises remain obscure. We hypothesized that epigenetic alterations may play a pivotal role in the molecular etiology of transgenerational genome instability.

Methods and Materials: We studied the levels of cytosine DNA methylation in somatic tissues of unexposed offspring upon maternal, paternal, or combined parental exposure.

Results: We observed a significant loss of global cytosine DNA methylation in the thymus tissue of the offspring upon combined parental exposure. The loss of DNA methylation was paralleled by a significant decrease in the levels of maintenance (DNMT1) and *de novo* methyltransferases DNMT3a and 3b and methyl-CpG–binding protein MeCP2. Along with profound changes in DNA methylation, we noted a significant accumulation of DNA strand breaks in thymus, which is a radiation carcinogenesis target organ.

Conclusion: The observed changes were indicative of a profound epigenetic dysregulation in the offspring, which in turn could lead to genome destabilization and possibly could serve as precursor for transgenerational carcinogenesis. Future studies are clearly needed to address the cellular and carcinogenic repercussions of those changes. © 2006 Elsevier Inc.

Radiation, Transgeneration genome instability, Epigenetics, DNA methylation, DNA damage.

INTRODUCTION

Studies have shown that ionizing radiation (IR) exposure has an indirect effect on genome stability, which could be transmitted through the germline of exposed parents to their progeny and could lead to genome destabilization and transgenerational carcinogenesis (1–3).

To date, a plethora of evidence has accumulated on the nature of transgenerational changes in the somatic tissues of the progeny of exposed parents—in particular, exposed fathers (1–3). Notwithstanding this, the exact molecular mechanisms leading to radiation-induced transgenerational genome instability and carcinogenesis are elusive and have lately been suggested to be epigenetic in nature (1, 2).

The global loss of DNA methylation at CpG dinucleotides was the first epigenetic abnormality identified in cancer cells (4) has recently been linked to the phenomenon of global genomic instability (1, 4).

METHODS AND MATERIALS

Animal model

In this study, sexually mature male and female C57BL/6 mice were randomly assigned to unexposed or exposed groups. Exposed

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This work was supported by an Alberta Cancer Board Grant to Olga Kovalchuk. Jonathan Loree was a recipient of The Natural Sciences and Engineering Research Council of Canada (NSERC) Undergraduate Student Research Award (USRA).

Acknowledgments—We thank Christine Picken and Igor Kovalchuk for their critical reading of this manuscript. We are grateful to Benjamin Thibault and Clayton Koganow for their technical assistance, to Karen Dow-Cazal and Charlotte Holmes for animal care, and to Franz Zemp for help with the statistical analysis.

Received April 12, 2006, and in revised form June 6, 2006. Accepted for publication June 6, 2006.

groups received 2.5 Gy of total body irradiation, 90kV, 5mA. Control groups were sham treated. Seven days after exposure the animals were randomly assigned to 4 mating groups, 4 breeding pairs per mating group. These were as follows: (*1*) animals with maternal exposure (exposed females mated to unexposed males); (*2*) animals with paternal exposure (unexposed females mated to exposed males); (*3*) animals with combined parental exposure; and (*4*) sham-treated animals serving as control group. No significant litter size differences were noted between the groups. The progeny were sacrificed at the age of 15 days.

Molecular analysis

DNA methylation analysis, DNA strand-break measurement, and western immunoblotting were conducted as previously described (5). Detailed experimental protocols are available in Supplementary Methods (http://people.uleth.ca/~olga.kovalchuk).

Statistical analysis

For the determination of the significance of the difference between the means, Student's *t* test with the post hoc Bonferroni correction was used. Analysis was performed using the JMP 5.0 and Excel XP software (Microsoft Corp., Redmond, WA).

RESULTS

To test whether changes in genome DNA methylation were observed in the somatic tissue of the offspring, global cytosine methylation was measured in the spleen, thymus, and liver of the offspring of all four mating groups using a sensitive HpaII-based cytosine extension assay (5, 6). We noted a DNA hypomethylation trend in the thymus tissue of the progeny upon paternal exposure, yet the trend was not statistically significant. On the other hand, combined parental exposure led to statistically significant ($p = 0.001049$) loss of DNA methylation in the thymus of offspring as compared with controls (Fig. 1a).

Having observed the loss of global genome methylation in thymus tissue of the progeny of exposed animals, we decided to elucidate the possible mechanism(s) of this phenomenon. The DNA methyltransferases DNMT1, DNMT3a, and DNMT3b are the three main functional enzymes that are responsible for setting and maintaining DNA methylation patterns in mammalian cells (7). We evaluated the effects of parental exposure on the expression of DNMT1, DNMT3a, and DNMT3b in thymus, spleen, and liver tissue of the progeny. DNMT1, DNMT3a, and DNMT3b expression was decreased in the progeny of exposed males or those progeny with combined parental exposure (Fig. 1b). In contrast to the thymus tissue, no DNMT expression changes were seen in either spleen or liver tissues.

The decrease in global cytosine DNA methylation, seen in thymus of the progeny upon combined parental irradia-

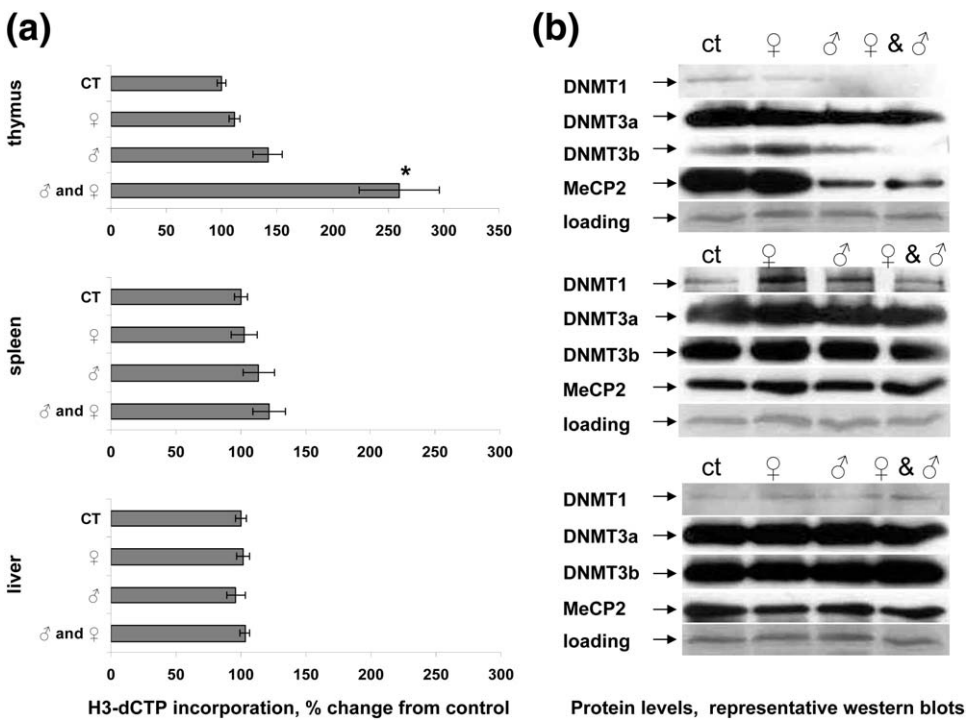

Fig. 1. Parental exposure leads to global genome hypomethylation and alters the expression of DNA methyltransferases and methyl-binding proteins in thymus of the offspring. (a) Levels of global genome DNA methylation. The extent of [^{3}H]dCTP incorporation is inversely proportional to the levels of methylation. Mean values ± SEM. Significant differences from the control animals are shown: *$p < 0.0125$ according to *post-hoc* Bonferroni correction. (b) Representative western blots. Each experiment included pooled lysates from 5 animals for each cohort, with equal representation of each animal. Western blots were repeated at least four times to ensure the reproducibility and robustness of the results. Ct = progeny of the control cohort; [female] = progeny of the maternal exposure group; [male] = progeny of the paternal exposure group; [male]+ [female] = progeny of the combined parental exposure group.