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COMMENTARY

LINE-1 in response to exposure to ionizing radiation

Igor Koturbash

Department of Environmental and Occupational Health, University of Arkansas for Medical Sciences, Little Rock, AR, USA

ABSTRACT

It is becoming increasingly recognized that Long Interspersed Nuclear Element, 1 (LINE-1), the most ubiquitous repetitive element in the mammalian genomes, plays an important role in the pathogenesis of disease and in the response to exposure to environmental stressors. Ionizing radiation is a known genotoxic stressor, but it is capable of targeting the cellular epigenome as well. Radiation-induced alterations in LINE-1 DNA methylation are the most frequently observed epigenetic effects of exposure. The extent of this aberrant DNA methylation, however, strongly depends on a number of factors, including the type and dose of radiation. Two other factors are being discussed in this commentary – the evolutionary age and type of the LINE-1 promoter, as well as the type of irradiated cell. This knowledge will further aid in elucidating the mechanisms of response to ionizing radiation exposure, as well in understanding the pathogenesis of the negative health effects associated with exposure.

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LINE-1 DNA methylation

Long Interspersed Nuclear Element, 1 (LINE-1) is the most ubiquitous transposable element in the mammalian genomes, accounting for approximately 17% of the human and 20% of the mouse genomes.[1] A typical mammalian LINE-1 element contains a 5′-UTR, two open-reading frames – ORF1 and ORF2 – and a 3′-UTR. While both ORFs are AT-rich, the 5′-UTR of LINE-1 elements in mammals is enriched in GC, with an average GC content of 57.2%.[2] This high density of GpC dinucleotides forms a heavily methylated CpG island in the promoter region of LINE-1. Methylation of LINE-1 DNA is considered among the primary mechanisms for its silencing and prevention of unwanted retrotransposition.[3]

Exposures to various environmental stressors have been shown to affect the DNA methylation status of LINE-1.[4] Alterations in DNA methylation may result in the loss of the epigenetic control over LINE-1 and lead to its transcriptional reactivation and retrotransposition. The latter event may lead to disruptive insertional mutagenesis when LINE-1 (most frequently – its 5′-truncated transpositionally inactive fragment) can be introduced within the ORF of the functional gene, leading to the aberrant function of the latter. LINE-1 DNA hypomethylation and retrotransposition have been associated with genomic instability and development of numerous pathological states, including cancer.[5] Even without retrotransposition, aberrant LINE-1 DNA methylation can substantially affect the tumor landscape. For instance, loss of DNA methylation in the intronic regions of MET, RAB3IP and CHRM3 proto-oncogenes within the fragments that owe to previous LINE-1 insertions leads to inadvertent activation of methylation-silenced genes, and is inversely correlated with metastasis-free survival and response to cancer therapy.[6,7]

LINE-1 and ionizing radiation

Ionizing radiation (IR) is a ubiquitous genotoxic stressor with recognized ability to alter the cellular epigenome. Exposure to IR often leads to the loss of global DNA methylation, which is attributed primarily to the loss of DNA methylation from repetitive elements and LINE-1 in particular (for a review, see ref. 8). This effect is mostly observed after exposures to doses of 1 Gy and above. At the same time, with the

CONTACT Igor Koturbash ikoturbash@uams.edu Dept. of Environmental and Occupational Health, University of Arkansas for Medical Sciences, 4301 W Markham Str, Little Rock, AR 72205, USA.

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growth of interest in radiation epigenetics, a number of studies have indicated that exposure to IR, especially at doses below 1 Gy, may also result in either an absence of changes in LINE-1 DNA methylation or even in DNA hypermethylation.[9–13]

To a certain degree, the observed discrepancies could be explained by the utilization of different models (*in vitro*, *in vivo*), doses and methods of analysis. Furthermore, it is becoming increasingly recognized that different types of IR may differentially affect LINE-1 DNA methylation. For instance, exposure to high-linear energy transfer (LET) IR, such as protons and heavy ions predominant in the space environment, often results in DNA hypermethylation.[14–16] We have previously shown that these effects originate primarily from repetitive elements, including LINE-1.[16]

In our two recent studies, we demonstrated that effects of IR on LINE-1 DNA methylation are also dependent on two other aspects: the evolutionary age and type of the LINE-1 promoter,[17] as well as on the type of irradiated cell.[18]

Ionizing radiation affects DNA methylation of selective LINE-1 elements

Patterns of the LINE-1 existence in mammals are characterized by the evolution of a single lineage of the elements. After the emergence and amplification to several hundreds or thousands of copies, the family is becoming extinct and being replaced by a new evolving family.[19] As recent works have shown, while ORFs of LINE-1 that belong to different families have a very high degree of homology, the major difference between the lineages stems from the UTR-regions.[2,19] In these regards, the 5′-UTR region is of particular interest, since besides its various functional units that regulate the expression of LINE-1, it contains a CpG island that is usually heavily methylated. At the same time, the vast majority of the analysis of LINE-1 DNA methylation was performed on the AT-rich ORF-1 and/or ORF-2, two open reading frames that are not only very conserved between the families, but which also contain only a small subset of CpG sites that can potentially be methylated. This bias towards the analysis of DNA methylation of ORF1 and ORF2 was probably due to the high complexity of the 5′-UTR and associated with that difficulties in the development of appropriate DNA methylation assays. The bias towards the ORFs has substantially minimized the capacity for the analysis of the LINE-1 DNA methylation.

In our recent study, we have shown that the effects of low-dose IR on LINE-1 DNA methylation are not uniform; that is, IR differentially affects DNA methylation of specific LINE-1 families, and this effect is dependent on their evolutionary age, at least in part.[17] In order to analyze the DNA methylation of LINE-1 families within their respective 5′-UTRs, we have developed an assay that is not dependent on the DNA bisulfite conversion and, thus, may successfully be applied to sequences with high CpG densities (for method description see, ref. 17).

First, we found that the DNA methylation status of LINE-1 elements is dependent on its evolutionary age. Specifically, the older the element was, the lower its DNA methylation within the 5′-UTR was. This effect was organ- and cell-type independent, as it was observed in the mouse lungs, as well as in the cells that belonged to various lineages of the mouse hematopoietic system. This can possibly be explained by the higher number of mutations in the evolutionary older elements due to the high rates of spontaneous deamination of 5-methylcytosine (5-mC). Another plausible explanation to this observed phenomena is that only the evolutionary younger elements are capable of retrotransposition and, thus, DNA methylation-mediated transcriptional silencing of the older elements would be redundant.

Next, we used two conventional approaches for evaluation of the ORF1 and ORF2 LINE-1 DNA methylation [methylation-sensitive real-time PCR (MS-RT PCR) and pyrosequencing], to evaluate the effects of exposure to low-mean absorbed doses of two types of densely IR – protons and heavy iron ions (^{56}Fe) on LINE-1. Independent of the approach or mode of exposure, we could not detect substantial changes in LINE-1 DNA methylation after irradiation. However, detailed examination of the 5′-UTR of the subset of LINE-1 elements that belonged to different promoter types and differed by evolutionary age clearly demonstrated the differential DNA methylation between the elements in response to IR exposure. While there were no changes in DNA methylation of evolutionary young elements (elements with evolutionary age up to 1.2 Myr), independent of the type of their promoters, substantial DNA hypermethylation was observed in the evolutionary older L1MdA_II element (evolutionary age 1.6 Myr). Mild to no increases in DNA methylation were observed in the 5′-UTR of the old LINE-1 elements (evolutionary age 4.7 Myr and older).

These results are in line with the other studies reporting DNA hypermethylation after exposure to densely IR.[16,20,21] The lack of DNA hypermethylation

in evolutionary younger elements (1.2 Myr and younger) can be explained by a relatively high degree of their initial DNA methylation within the 5′-UTR and inability to accept additional methyl groups. The lack of IR-induced DNA hypermethylation in the evolutionary older elements (4.7 Myr and older) can potentially be explained by the decreased number of CpG sites capable of accepting methyl groups due to the process of spontaneous deamination of 5-mC mentioned above. The nature of the densely IR-induced LINE-1 DNA hypermethylation, however, remains unknown and warrants further investigation.

Ionizing radiation affects LINE-1 DNA methylation in target cells

Over 200 cell types that build the mammalian organisms share the same genomic blueprint; yet, they are phenotypically distinct and comprise distinct organs and tissues with highly specialized functions. Although all the organs and systems are known to be hit by IR equally, some are considered "target" organs. The hematopoietic system is among the most sensitive to IR and is comprised of cells of different lineages. Among the latter, hematopoietic stem cells (HSCs) represent the most sensitive to IR compartment. Given that HSCs reside at the top of the hematopoietic system hierarchy, IR-induced damage to them predetermines the negative long-term sequelae of the exposure.

In our most recent study, we addressed the differential DNA methylation of LINE-1 elements in the mouse hematopoietic system two months after total body irradiation (TBI) to low-dose IR (0.1 and 1 Gy of ^{137}Cs) in relatively radioresistant C57BL/6J and radiosensitive CBA/J mice.[18] The latter are also known as a model for the studies on radiation-induced leukemia. We have shown that DNA methylation of LINE-1 elements was affected in a cell lineage- and mouse strain- and dose-dependent manner. Specifically, we demonstrated that TBI resulted in the loss of LINE-1 DNA methylation selectively in HSCs – the cells characterized by the highest sensitivity to IR. At the same time, minor changes were observed in the hematopoietic progenitor and mononuclear cells (HPCs and MNCs, respectively). These effects were observed in the hematopoietic system of CBA/J mice, while no significant TBI-induced changes in LINE-1 DNA methylation were observed in the hematopoietic system of C57BL6/J mice.

Loss of global/LINE-1 DNA methylation is not only a generally-recognized hallmark of cancer, but may also play a driving role in carcinogenesis.[22–24] Indeed, LINE-1 DNA hypomethylation was reported as a result of exposure to various carcinogens in experimental settings and in pre-malignant conditions in human patients.[5,24] The exact mechanisms of how LINE-1 DNA hypomethylation contributes to carcinogenesis are unknown. However, it was recently shown that DNA hypomethylation of the LINE-1 insert-containing proto-oncogenes may reactivate the latters.[6,7] Furthermore, it was proposed that DNA hypomethylation-induced activation and subsequent retrotransposition of LINE-1 may result – aside from genome amplification – in the development of genomic instability (GI).[11] Radiation-induced GI is a multifactorial phenomenon exhibited as an increased frequency of mitotically heritable genetic alterations that are observed in the unirradiated progeny of irradiated cells multiple generations after exposure.[25,26] Although no studies to date investigated the role of LINE-1 in the development of radiation-induced GI, exposure to IR has been shown to stimulate LINE-1 retrotransposition.[27,28] Importantly, in our study, LINE-1 DNA hypomethylation in the hematopoietic stem cells of radiosensitive CBA/J mice was observed two months after irradiation and was not associated with any detectable damage to DNA, absence of increase in ROS and cellular senescence.[18] Altogether, these findings support the hypothesis that IR may cause persistent epigenetic alterations in the target tissues/cells and that these alterations may serve as driving mechanisms of IR-induced carcinogenesis.

Concluding remarks and future prospects

The results of these studies may aid in better understanding the mechanisms of response and pathogenesis of the effects of exposure to IR. While hundreds of thousands of copies of LINE-1 may be present within the mammalian genome, identification of specific LINE-1 families affected by the exposure may help to identify the particular set of genes that are targeted by IR. This set of genes would include the genes that have evolutionary acquired LINE-1 inserts or belong to a recently described adjacent to LINE-1 DNA modules.[29,30] Further identification of target cells within the exposed organs/systems may further help in understanding the health effects of exposure and development of potential mitigating strategies.

Besides being an excellent tool for the studies on radiation epigenetics, LINE-1 holds the promise for

the development of a biomarker of previous exposure (s), since irradiation leads to long-term/persistent alterations in the pool of target cells. Of particular interest, in these regards, is the analysis of LINE-1 DNA methylation in peripheral lymphocytes that may serve as a non-invasive biomarker as has been previously shown in the rat model.[31]

Abbreviations

5-mC	5-methylcytosine
CpG	CG sequence in the genome
GI	genomic instability
Gy	gray
IR	ionizing radiation
HPCs	hematopoietic progenitor cells
HSCs	hematopoietic stem cells
LET	linear energy transfer
LINE-1	long interspersed nucleotide element, 1
MNCs	mononuclear cells
Myr	million years
ORF	open reading frame
TBI	total body irradiation
UTR	untranslated region

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ORCID

Igor Koturbash http://orcid.org/0000-0002-4090-7008

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