Epigenetic Memory of Space Radiation Exposure

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Manned interplanetary travel is imminent, but currently inhibited not only by factors such as technology and budget, but also by the uncertainty surrounding the health risks associated with galactic cosmic radiation (GCR) exposure, for which there is currently no effective means of shielding. Much research has focused on the influence of high energy and charge (HZE) nuclei, the most detrimental component of GCR, on the genome. Exposure to radiation, either terrestrially or in space, induces DNA damage that, if not accurately repaired, can give rise to genetic mutations with long-term biological implications including development of chronic diseases, such as cardiovascular disease and cancer. The radiation encountered on Earth is composed primarily of low linear energy transfer (low-LET) photons (eg. gamma rays or X-rays) that react only sparsely with cellular components, with DNA damage arising primarily from free radicals generated by the ionization of nearby water molecules. In contrast, HZE ions have a high linear energy transfer (high-LET) and deposit energy linearly along the particle trajectory, interacting directly with macromolecules in addition to giving off high energy electrons (δ rays) that extend laterally for several microns. This creates a more dense ionization track as the particle moves through the cell and nucleus and results in a more complex DNA damage that involves mixtures of more than one type of DNA damage in close proximity (double strand breaks, single strand breaks, base damage, etc) (Cucinotta and Durante, 2006; Durante and Cucinotta, 2008). This clustering of multiple types of DNA damage is thought to pose a challenge for the DNA repair machinery, potentially accounting for the more deleterious biological effects of high-LET radiation than low-LET radiation at similar doses. High-LET radiation exposure can also have a lasting impact on cellular physiology without any DNA mutation, via alterations in DNA methylation, though the frequency and consequence of these

changes has not been well-documented.

DNA Methylation

DNA methylation refers to a chemical modification of cytosine bases in DNA. In most cells this modification occurs at adjacent cytosine-guanine nucleotides called CpG sites. DNA methylation influences which genes are expressed and in what context, allowing for a diverse collection of cell types with distinct functions to arise from a single common genome. DNA methylation is thus considered an "epigenetic" modification, as it represents another level of information that sits "on top of" the genetic code (the DNA sequence) and influences how the genetic code is interpreted (Baylin and Jones, 2011). Most CpG sites in the genome are methylated, including the regions within and between genes. Methylation in these intergenic regions plays an important role in the silencing of mobile elements (transposons) and in maintaining chromosome structure and stability. Also embedded within these methylated regions are 'enhancers', long-range regulatory elements that can influence the expression of genes from a great distance. In contrast, a small fraction of the genome is composed of areas dense with CpG sites, called CpG 'islands', that typically remain unmethylated in normal cells. More than half of human protein coding genes and many non-coding RNAs are regulated by promoters that lie within CpG islands. A small subset of CpG island promoters acquire methylation normally during development and cell-type specification as part of a program to ensure the long-term silencing of the neighboring gene (for example, during the programmed silencing of one X-chromosome in female mammals). Such epigenetic regulation of gene expression is critical to normal development and plays an important role in the maintenance of

cellular identity. However, unlike DNA sequence, DNA methylation patterns change readily over time and with normal aging, and represent an important feature of how organisms adapt to a changing environment (Huidobro et al., 2013; Jones, 2012).

DNA methylation patterns can change in response to extrinsic environmental factors (e.g. nutrition, chemical pollutants) and with age (Breitling et al., 2011; Mirbahai and Chipman, 2014). Because DNA methylation patterns are copied along with the DNA sequence during cellular replication, induced changes to the epigenetic state will persist over multiple cell divisions, resulting in a lasting and mitotically heritable "memory" of prior exposures. Such induced alterations to DNA methylation have the potential to contribute to the long-term health risks associated with radiation exposure. The consequence of DNA methylation changes will depend on their genomic context. For example, loss of methylation outside of CpG islands occurs during aging and cancer progression and can lead to aberrant activation of transposons, chromosome instability, and (potentially) to the activation of cryptic enhancers (Taberlay et al., 2014). Methylation in the transcribed regions of genes is positively correlated with gene expression, and depletion in these areas could result in the reduced expression of some genes (Lister et al., 2009). Conversely, the aberrant gain of methylation in normally unmethylated CpG islands is associated with gene silencing and has been shown to contribute to cancer formation through the stable and heritable silencing of important growth suppressor genes (reviewed in Baylin and Jones, 2011).

Effects of Radiation on DNA Methylation

Most research to date assessing the impact of radiation exposure on the epigenome has

focused on the effects of low-LET X-rays on global methylation trends. Nearly all of these studies report global hypomethylation in response to relatively high doses of X-rays (eg. up to 10 Gy) (Antwih et al., 2013; Aypar et al., 2011; Filkowski et al., 2010; Goetz et al., 2011; Ilnytskyy et al., 2009; Pogribny et al., 2004). It has been proposed that the global hypomethylation is a consequence of the inability of the maintenance DNA methyltransferase, DNMT1, to keep up with the newly synthesized DNA generated during the repair of massive DNA damage (Pogribny et al., 2004), although decreased expression of DNMT1 itself and increased expression of miR-29, which negatively regulates methyltransferase expression, have also been reported (Antwih et al., 2013; Filkowski et al., 2010).

Considering the unique characteristics of the high-LET radiation track and the damage it elicits, there is the potential for unique effects on the epigenome. Indeed, current research indicates that exposure to high-LET radiation can also result in lasting changes in the total levels of DNA methylation in the genome, and that those changes may be different from the DNA methylation changes seen in response to equivalent doses of low-LET radiation (Aypar et al., 2011; Goetz et al., 2011; Lima et al., 2014; Nzabarushimana et al., 2014). Although there is some disagreement among the few studies that have assessed the effects of high-LET and HZE radiation on the epigenome, the majority indicate a trend toward global hypermethylation (Antwih et al., 2013; Goetz et al., 2011; Lima et al., 2014; Nzabarushimana et al., 2014).

Outstanding Questions

Does radiation exposure cause DNA methylation changes at specific regions of the human genome, like genes or other genomic compartments? Are the methylation changes that

occur with radiation exposure random, or are there regions that are more prone to radiation-induced methylation 'damage'? Few studies have assessed the effect of high-LET radiation at specific CpG sites. While CpG sites in the promoters of a few select genes were tested in the above studies, no consistent alterations were observed. Methods are now in place to study methylation at essentially all 28 million CpG sites (Lister et al., 2009), allowing the complexity and target specificity of CpG methylation changes across the entire human genome to be explored.

Given that DNA damage can precipitate local changes in DNA methylation (Morano et al., 2014; O'Hagan et al., 2008) and DNA methylation changes are known to occur with aging, and to contribute to the progression of cancer and other diseases (Teschendorff et al., 2010), it is tempting to speculate that such epigenetic 'scars' could contribute to the long-term effects of radiation exposure even if the initial damage to the DNA is ultimately repaired (Figure 1). Support for this idea stems from studies by O'Hagan (O'Hagan et al., 2008) and Morano (Morano et al., 2014) who showed that DNA double stranded breaks, which are characteristic of high-LET radiation, can result in stable DNA methylation-mediated transgene silencing after successful break repair. Alternatively, the observed changes in DNA methylation may reflect an indirect consequence of the broader cellular stress response. Indeed, exposure to reactive oxygen species associated with chronic inflammatory states are known to contribute to cancer risk, and can precipitate lasting changes in DNA methylation and chromatin modifications (Hahn et al., 2008; O'Hagan et al., 2011). Elevated reactive oxygen species can persist for up to two weeks after high LET radiation exposure (Werner et al., 2014) and could at least in principle contribute to an altered epigenome (Figure 1).

Future Perspectives

While much has been learned about the functions and significance of DNA methylation over the last several decades, our current understanding is undergoing rapid revision. The recent discovery that endogenous methylated cytosine residues in mammalian DNA are naturally subject to enzyme-driven oxidation and detection of the oxidized methylcytosine derivatives (hydroxymethylcytosine (hmC), formylcytosine (fC), and carboxycytosine (caC)) in human and mouse DNA has prompted considerable effort to decipher the relative roles of these modified residues in gene expression and genome function (reviewed in Wu and Zhang, 2014). The fC and caC forms serve as substrates for the DNA repair machinery, which removes the oxidized base and replaces it with unmodified cytosine, resulting in a net "demethylation" event, suggesting that DNA methylation patterns may be far more dynamic than previously anticipated. How radiation exposure of any type influences the formation or removal of these modified methylcytosine bases has not yet been explored. Furthermore, DNA methylation patterns are only one component of a broader epigenetic 'code' that includes posttranslational modifications to the histone proteins on which the DNA is wound into chromatin. Together, the pattern of DNA methylation and histone modifications helps to organize the genome into domains of different transcriptional potential. While local changes in histone modification status at the site of radiation-induced double strand breaks is known to play an important role in alerting the DNA repair machinery and cell cycle to the presence of DNA damage (Price and D'Andrea, 2013), recent work has suggested that radiation exposure (X-ray) can also influence global levels of various modified histones, suggesting a broader reprogramming of the epigenomic landscape (Maroschik et al., 2014). Methods exist to map the genome-wide

patterns of histone modifications as well, and have not yet been applied to high LET radiation exposure. Indeed, it has been argued that an assessment of DNA methylation and other epigenetic modifications should be considered in environmental toxicity and risk assessment, and that an understanding of the specific epigenetic "footprint" left by various chemical or physical toxins could one day be used to monitor a person's exposure history (Mirbahai and Chipman, 2014). The identification of specific and unique DNA methylation changes associated with high-LET radiation and known to be associated with diseases such as cancer, could in principle be used by NASA for 'biodosimetry'; monitoring the biological impact of cumulative high-LET radiation exposure and the associated health risks encountered by astronauts in deep space (Cucinotta, 2014).

The technology and informatics to address our unanswered questions are available and affordable. Array-based platforms capable of analyzing >480,000 CpG sites (Illumina Human Methylation 450K array) have been available for several years now and have been applied to the study of DNA methylation during aging and in various disease states including cancer (Fang et al., 2011; Florath et al., 2014; Liu et al., 2013; Noushmehr et al., 2010). DNA methylation profiles are now available for thousands of human tumors from more than 25 different tumor types as part of the NIH-funded the Cancer Genome Atlas (TCGA) Project (https://tcga-data.nci.nih.gov/tcga/) and other similar international consortia (the International Cancer Genome Consortium; https://dcc.icgc.org/) and a wealth of information exists in the public domain for comparative studies. Next-generation sequencing-based methods allow for the analysis of DNA methylation at single base pair resolution, allowing for even greater genome coverage. For all of these approaches, robust statistical methods are necessary and are

continuously being developed to address potential issues such as proper normalization strategies (Wu et al., 2014), cell type or tissue heterogeneity (Houseman et al., 2012), adjustment for other confounding factors such as age or population stratification (Barfield et al., 2012, 2014), or the low number of replicates typically available in sequencing studies (Feng et al., 2014) . The questions we seek to answer about the effects of GCR involve larger questions about the functions of methylation in our genome. As we move forward to answer these questions we will not only step toward a future of space travel, but toward a greater understanding of our own biology.

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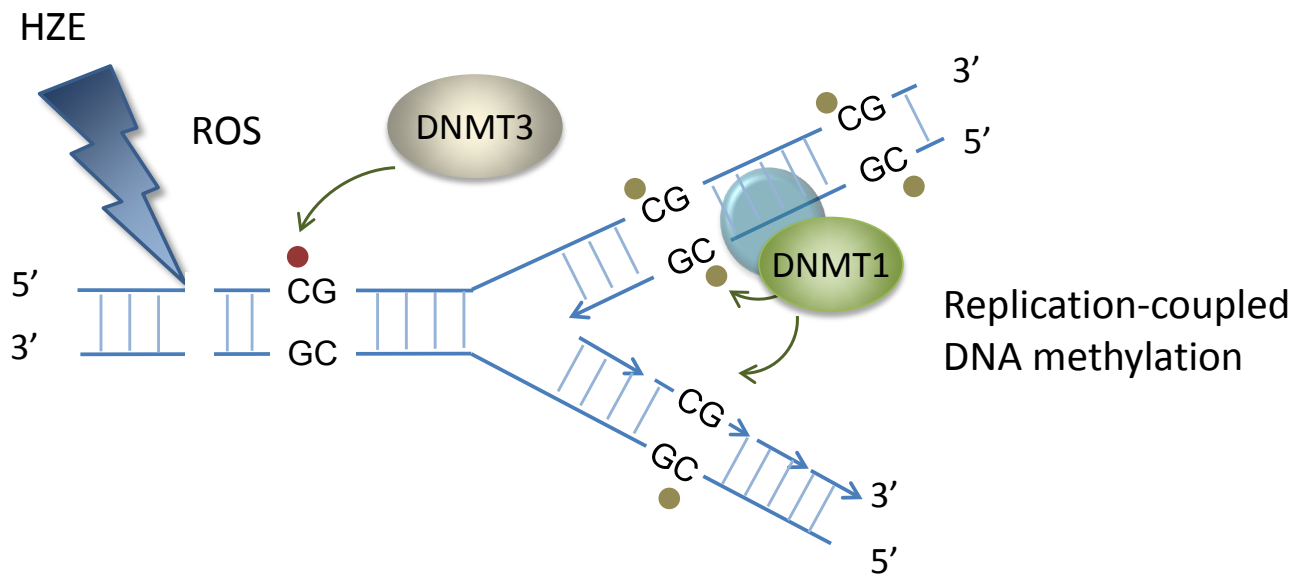

Figure 1 – Epigenetic alterations provide a long-term memory of prior space radiation exposure.

Alterations in DNA methylation resulting from acute radiation exposure and ensuing DNA damage or persistent reactive oxygen species (ROS) have the potential to become "fixed" if they are subsequently replicated, leading to heritable epigenetic re-programming. DNA methylation (gold dots) occurs primarily at CG residues in the genome. The pattern of DNA methylation is copied during DNA replication by DNA methyltransferase 1 (DNMT1) that recognizes the methylated CG on the parental strand and transfers a methylgroup to the cytosine on the newly-synthesized strand, thereby preserving the methylation patterns in daughter cells. Gains or losses in DNA methylation induced by acute radiation exposure will likewise be copied to subsequent cell generations in the next mitosis. DNA damage, such as double strand breaks, can serve as a stimulus for a new methylation mark (red dot), and could leave an epigenetic "scar" even if the break is successfully repaired.

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