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Authors: Hunt, Clayton R., and Pandita, Tej K.

Source: Radiation Research, 192(6) : 577-578

Published By: Radiation Research Society

URL: https://doi.org/10.1667/RR15541.1

RADIATION RESEARCH **192**, 577–578 (2019)
0033-7587/19 $15.00

DOI: 10.1667/RR15541.1

COMMENTARY

"What's Past is Prologue": Pre-Existing Epigenetic Transcriptional Marks May Also Influence DNA Repair Pathway Choice

Clayton R. Hunt and Tej K. Pandita[1]

The Houston Methodist Research Institute, Houston, Texas 77030

Understanding the precise mechanisms involved in the repair of ionizing radiation- and chemotherapeutic drug- is an essential step in the targeted development of new and improved cancer treatments. Once understood, each step in the repair process presents a potential point of intervention. Recent studies have established that different chromosomal regions are prioritized for repair, in particular active gene regions as opposed to inactive or condensed regions (*1*). These two regions are characterized by different epigenetic signatures leading to the suggestion that pre-existing chromatin modifications should be important determinants of DNA damage repair and, by extension, potential targets for clinical intervention.

Chromatin is subject to a wide range of covalent modifications that serve as biological marks for various functions. The acetylation of histone H4 at lysine 16 (H4K16ac) is a well characterized dynamic mark associated with active gene transcription (euchromatin) (*2*). Moreover, H4K16ac has been shown to produce an open chromatin conformation that should allow greater DNA access for both transcription factors and repair factors.

Taking advantage of previous mapping data as well as their own H4K16ac genomic site map, Horikoshi *et al*. (*1*) have combined this data with a CRISPR/Cas9 approach to introduce a single 25 bp oligonucleotide element containing a I-Sce1 restriction site into unique sequence and H4K16ac defined genomic sites. Similarly, the NHEJ repair (EJ5-GFP) and the HR repair (DR-GFP) cassettes (*1*) were also inserted by CRISPR/Cas9 at specific sites allowing for specific repair pathway measurements based on appropriate re-constitution of the disrupted GFP gene (*1*). The major advantages of this approach are twofold: First, it allows for detailed examination of the repair process at a single site, not as the average of multiple undefined sites typically induced by radiation or DNA damaging drugs; Secondly, by using CRISPR/dCas9-mediated localization of chromatin modifying enzymes it is possible to increase local modification levels and measure the effect on the repair mechanism. This technique initially developed by Wang *et al*. (*3*) and then by Horikoshi *et al*. (*1*) uses a dCas9-MOF fusion protein to increase H4K16ac levels at specific sites. Surprisingly, though this approach increased H4K16ac levels at all targeted sites but increased DSB repair only at a site in a transcribing DNA region, not at one in a non-transcribed region.

Examination by Horikoshi *et al*. (*1*) of a number of I-Sce1 induced DSBs and repair cassettes inserted at defined chromosomal sites revealed that the pre-DSB H4K16ac regional status had a major influence on repair pathway choice. Sites within H4K16ac rich regions characteristic of gene-rich/transcriptionally active euchromatin regions preferentially used the HR pathway as compared with heterochromatin/H4K16ac poor sites (Fig. 1). Moreover, inhibition or depletion of RNA polymerase or the Cockayne syndrome B chromatin remodeling/helicase protein decreased HR repair factor recruitment to the DSB sites, further confirming the relationship between pre-existing H4K16ac levels, transcription and HR mediated DNA damage repair.

Beyond these primary findings, the conceptual approaches of Horikoshi *et al*. (*1, 2*) suggests DNA damage repair studies will now enter a new sphere in which extensive epigenetic modification maps are combined with CRISPR/Cas9 mediated introduction of targeted DSBs and the ability to modulate local chromatin modification levels to measure their effect on repair mechanisms (*1*). This new ability to examine site specific and modification specific effects on repair are likely to yield unexpected results that could potentially impact radiation and chemo-based cancer therapies.

Received: October 10, 2019

[1] Address for correspondence: The Houston Methodist Research Institute, Smith Building 8.026, 6550 Fannin St., Houston TX 77030: e-mail: tpandita@houstonmethodist.org.

References

1. Horikoshi N, Sharma D, Leonard F, Pandita RK, Charaka VK, Hambarde S, et al. Pre-existing H4K16ac levels in euchromatin

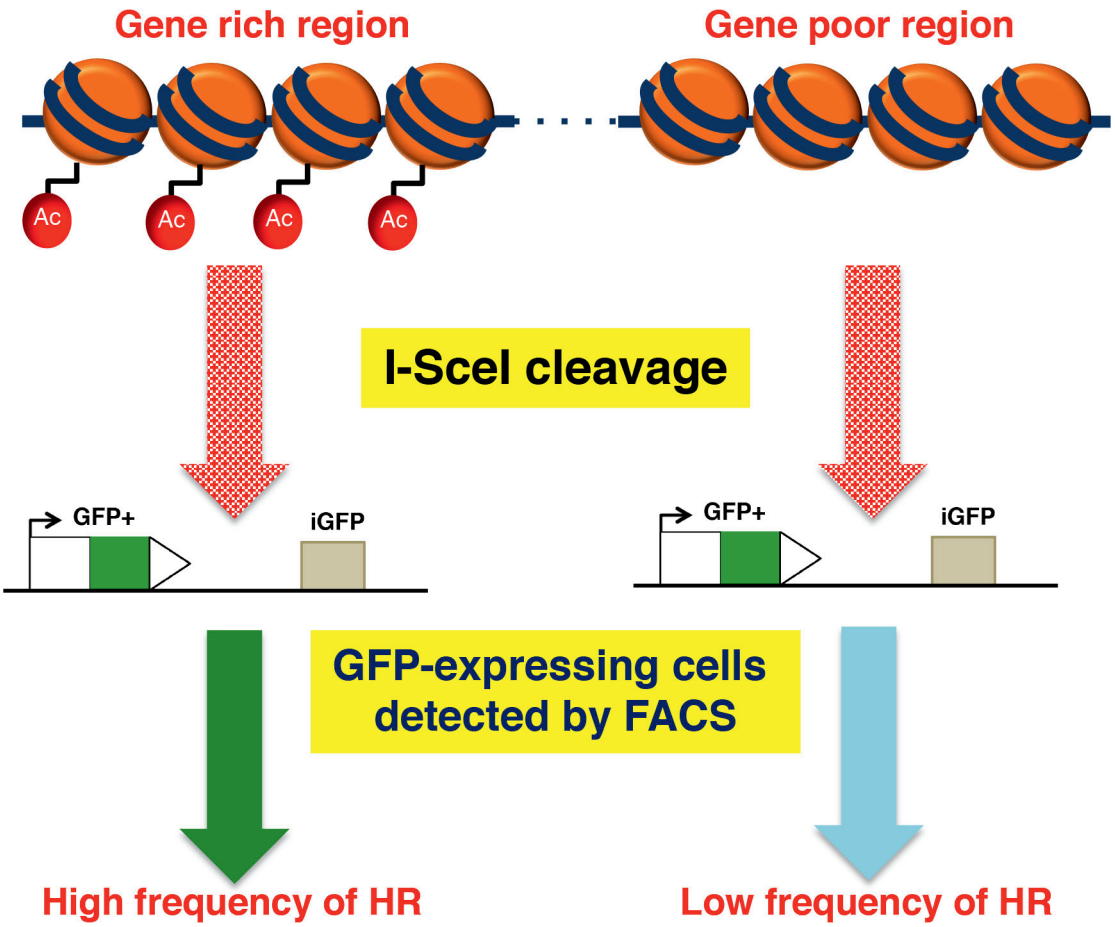

FIG. 1. DNA DSB repair of homologous recombination (HR) cassettes in regions with pre-existing high and low histone H4 lysine16 acetylation (H4K16ac) at gene rich and -poor regions of human chromosomes. Gene rich regions with high levels of H4K16ac have high frequency of DSB repair by homologous recombination.

drive DNA repair by homologous recombination in S-phase. 2019 Commun Biol 2:253. doi: 10.1038/s42003-019-0498-z

2. Horikoshi N, Kumar P, Sharma GG, Chen M, Hunt CR, Westover K, et al. Genome-wide distribution of histone H4 Lysine 16 acetylation sites and their relationship to gene expression. 2013 Genome Integr 4:3. doi: 10.1186/2041-9414-4-3

3. Wang T, Wei JJ, Sabatini DM, Lander ES. Genetic screens in human cells using the CRISPR-Cas9 system. 2014 Science 343:80–4. doi: 10.1126/science.1246981