Chromatin Regulation and Low Dose Irradiation: A Two-Way Street ?

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Goal: We are trying to understand the impact of exposure to low-dose ionizing radiation on epigenetic mechanisms, chromatin organization and functional responses, and how these processes affect the response to low-dose radiation.

Background/Significance: Genomic instability (GI) is one of the hallmarks of cancer that contributes to genetic diversity and has been associated with exposure to ionizing radiation (IR) (1,2). Radiobiology dogma has focused on DNA as the likely target for GI effects of radiation, but until fairly recently, without incorporating roles for chromatin. Eukaryotic genomes contain two major chromatin domains: heterochromatin and euchromatin. Heterochromatin is enriched for highly repetitive sequences and specific epigenetic marks and proteins, and represents a specific challenge for double-strand break (DSB) repair. This cytologically-distinct region of the nucleus comprises about 30% of fly and human genomes. The large number of repeated sequences in heterochromatin, and their close proximity within nuclei, exacerbates the risk for genome rearrangements in the presence of DSBs, particularly during homologous recombination (HR) repair. Recombination between identical repeats on non-homologous chromosomes produces dicentric and acentric chromosomes, known to contribute to human diseases such as cancer and infertility. An unresolved question is whether DSBs in heterochromatin are repaired by non-homologous end joining (NHEJ) or HR, and how repair occurs without threatening the stability of the genome. Previous research has demonstrated that heterochromatin packaging and proteins are required to assure genome stability (3). How heterochromatin components promote genome stability, and whether they regulate DSB repair, are the focus of our research.

Approach: Using fixed and live *Drosophila* cells, we have tested three possible mechanisms to determine how heterochromatin components promote genome stability, and whether they regulate repair of DSBs: (a) since γH2AX foci form preferentially in actively transcribing euchromatin, and form less frequently in heterochromatin following gamma irradiation (4), compaction or chromatin composition could make heterochromatin intrinsically less responsive to DSB formation or processing, (b) heterochromatin components could repress homologous recombination (HR) and promote less-damaging repair processes, similar to suppression of reciprocal recombination in pericentric heterochromatin during meiosis, which requires heterochromatin components, or (c) HR repair could occur in heterochromatin, but aberrant recombination is prevented by previously unidentified mechanisms.

Results: We show that IR-induced DSBs are efficiently formed and processed in *Drosophila* heterochromatin, and that their repair is surprisingly dependent on HR. We demonstrate that heterochromatin responds dynamically to IR in two ways. First, the heterochromatin domain expands immediately after IR, and displays dynamic protrusions into the euchromatin domain. Second, proteins involved in early stages of HR (e.g. ATRIP) are quickly recruited to DSBs, then repair sites display a dramatic relocalization to outside heterochromatin, where they first recruit Rad51, which is required for strand invasion and completion of HR repair. We show that resection and

checkpoint proteins are required for both heterochromatin expansion and DSB relocalization, whereas Rad51 is only required for relocalization. In addition, we identified the Smc5/6 complex as a new heterochromatin component that serves as a key regulator of HR repair in time and space.

Conclusion/Future plans: Our work reveals that DSBs are formed in Drosophila heterochromatin after exposure to ionizing radiation and are rapidly recognized and processed for repair. Intriguingly, we find that DSBs in heterochromatin move outside of a dynamic heterochromatin domain to complete recombinational repair (5). We propose that the spatial and temporal control of DSB repair in heterochromatin, especially the separation of early and late HR events, safeguards genome stability by preventing aberrant exchanges between repeats. Notably, we have shown that heterochromatin expansion occurs after low-dose radiation in Drosophila, and are now focused on investigating the relevance of heterochromatin dynamics to the low-dose response.

Direct support for the DSB movement to the periphery of heterochromatin compartments in the repair of heterochromatic DNA lesions has also been recently reported in mammalian cells (6). We are currently determining if the other aspects of the response of heterochromatin and heterochromatic DSBs identified in Drosophila occur in mammalian cells after low-dose radiation, and whether they impact low-dose responses. We are also investigating whether regulators of these responses identified in Drosophila play the same roles in mammalian cells.

In addition, we will briefly highlight work from the LBNL Low Dose SFA that has elucidated other important aspects of the relationship between low-dose radiation and chromatin structure/function, which are described in more detail in the poster/abstracts presented by Sylvain Costes (Non-linear DNA damage response to low-dose ionizing radiation suggests clustering of DNA breaks in normal human cells) and Terumi Kowhi-Shigematsu (Impact of Low-Dose Ionizing Irradiation on Histone Modification and Chromatin Organization).

References

1. Hanahan D and Weinberg RA (2011) Hallmarks of cancer: The next generation, ***Cell*** 144(5): 646-674.
2. Baskar R (2010) Emerging role of radiation induced bystander effects: Cell communications and carcinogenesis, ***Genome Integrity*** 1(1):13.
3. Peng, J. and Karpen, G.H. (2008). Epigenetic regulation of heterochromatic DNA stability. ***Current Opinion in Genetics & Development***. 18, 204-11.
4. Kim, J.-A., Kruhlak, M., Dotiwala, F., Nussenzweig, A., and Haber, J.E. (2007). Heterochromatin is refractory to gamma-H2AX modification in yeast and mammals. ***J Cell Biol*** *178*, 209-218.
5. Chiolo I, Minoda A, Colmenares SU, Polyzos A, Costes SV and Karpen GH (2011), ***Cell*** 144 (5):732-744.
6. Jakob, B., Splinter, J., Conrad, S., Voss, K.-O., Zink, D., Durante, M., Löbrich, M. and Taucher-Scholz, G. (2011). DNA double-strand breaks in heterochromatin elicit fast repair protein recruitment, histone H2AX phosphorylation and relocation to euchromatin. ***Nucleic Acids Research*** doi:10.1093/nar/gkr230.

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