Impact of Low-Dose Ionizing Irradiation on Histone Modification and Chromatin Organization

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Goal

Our goal is to understand the effect of low-dose ionizing radiation on the chromatin of mammalian cells, focusing on epigenetic modifications of histone and three-dimensional organization of chromatin, involving looping.

Background and Significance

The Project 3 in Berkeley Lab's SFA addresses both fundamental problems in gene expression as well as the effects of low dose on chromatin organization leading to functional significance. Both epigenomic modification of chromatin and chromatin organization affect gene expression. Therefore, it is important to learn if chromatin structure is altered by low-dose ionizing radiation. Such alteration in chromatin structure may subsequently lead to either resistance to other insults or alternatively lead to aberrant function and mutation leading to cancer

Approach and Results

We have chosen both a non-malignant human breast cell line, MCF10A, and thymocytes from a model organism (mouse). Here we report on the results obtained with the human breast cell line, and with mice thymocytes. We subjected these cells to a low dose of 0.1Gy radiation. After 20 min, we performed ChIPseq analysis (chromatin immunoprecipitation followed by high-throughput sequencing) using antibody against histone H3 lysine 4 trimethylation (H3K4me3), a histone mark associated with transcriptionally active chromatin. This particular modification of H3 was found to be dramatically increased genome-wide 20 min after irradiation. Although H3K4me3 is known to mark promoter regions of transcriptionally-active genes, the modification was found also in transcriptionally silent genes after irradiation. Having verified this important finding we now have expanded our study by using another mark, H3K36me3, that is known to modify coding regions of active genes. The deep sequencing is currently underway and the results should become available soon.

In addition to histone modification, we studied the effect of ionizing radiation on chromatin looping. Chromatin looping is another example of epigenetics, because the way that chromatin is packaged determines inducibility of some genes, and is involved in fine-tuning the levels of expression of genes that are interacting in the nuclear space, without changing the primary DNA sequence. We recently established a method in mammalian cells to capture chromatin looping events (interactions of one specific site of choice with all other sites in the genome mediated by a protein of interest referred to as ChIP-one-to-all). The final step in this chromatin-looping assay that determines intra- and inter-chromosomal interactions is high-throughput sequencing. This chromatin-looping assay is now carefully optimized so that the *c-Myc* gene interaction partners could be detected reproducibly and robustly among independent biological replica of thymoyctes isolated from different mice. Given the complexity of the assay and in order to set up all parameters to be able to measure subtle changes, we used 0.1 as well as 1Gy of irradiation. Our

preliminary data show that 20 min post 1Gy of irradiation, chromatin-looping events across different chromosomes (inter-chromosomal looping) are greatly reduced and the looping events are constrained mainly to intra-chromosomal looping in relation to the sham irradiated control cultures. The deep sequencing for chromatin looping studies with thymocytes exposed to 0.1Gy radiation is currently underway. During the first 20 min after irradiation, while chromatin looping and epigenetics are undergoing major changes, the DNA damage sites marked by γ-H2AX are found to be recruited to the SATB1 regulatory network in thymocytes. We have shown that SATB1 promotes assembly of γ-H2AX foci with DNA repair proteins; the frequencies of the assembly were assessed by a new software developed by Sylvain Costes. In the absence of SATB1, the DNA repair process in thymocytes is much delayed, presumably because there is no nuclear architecture to facilitate formation of the DNA repair complex.

Conclusion and Future Plan

Our ChIPseq data show that chromatin exposed to low-dose ionizing radiation exhibits epigenetic marks known to be associated with transcriptionally-active chromatin, indicating a transition to the "open" state of chromatin conformation genome-wide. This phenomenon is not necessarily accompanied by transcriptional activation, because silent genes remained silent, and yet these genomic loci were marked by transcriptionally-active histone modifications. Chromatin looping events also exhibited changes upon irradiation, and they become mostly restricted to intra-chromosomal looping as opposed to inter-chromosomal looping by radiation. These dynamic changes in chromatin conformation are most likely necessary for DNA repair processes. The nuclear architecture protein SATB1, which mediates chromatin looping, also functions as a platform for assembly of DNA repair factors to promote the repair process after irradiation. The main questions to address in the following year are to determine the biological consequences of these initial chromatin changes after irradiation in human breast cells, and to determine those signaling factors that influence such changes.

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