Genome-wide Changes in Epigenomics Induced by X-irradiation

Hunter W. Richards, Steven D. Ayers, Shutao Cai, Jingchuan Li, Yoshinori Kohwi, Paul Yaswen, Gary Karpen, Sylvain Costes and Terumi Kohwi-Shigematsu

Life Sciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, 1 Cyclotron Rd., Bldg 84-155, Berkeley, CA, USA, 94720.

After radiation insult, sites of damaged DNA are marked by phosphorylation of S139 ofH2AX (γH2AX). In addition to γ-H2AX modification, there may be other changes in chromatin structure at the level of histone modifications in response to ionizing radiation. By chromatin immunoprecipitation-quantitative PCR (ChIP-qPCR), we examined histone modifications at both transcriptionally-active and -silent gene loci in mammalian cells after they were exposed to x-irradiation at different doses. In mouse thymocyte, we found a significant increase in histone H3 K9/14 acetylation levels, histone marks corresponding to active chromatin, in these gene loci. The increase in the histone modification mark is transient, and returns to the normal level by 6 hrs after irradiation. However, while H3K9/14 acetylation levels were increased in transcritionally-inactive gene loci which we examined, these genes remained repressed after x-irradiation. On the other hand, expression of genes known to be induced by irradiation was found to be increased as expected. Therefore, our data strongly suggests a global change in chromatin conformation to an “open” configuration induced by x-irradiation independent of transcription.

By Illumina GAII sequencing (ChIP-seq, using antibodies against modified H3, we further examined x-irradiation-induced epigenomic changes at the genome-wide scale. The ChIP-seq data were generated for immortalized human breast epithelial cells, MCF10A cells, with an antibody against trimethylated histone H3K4 (H3K4me3), another histone mark corresponding to active chromatin, at various doses (0Gy, 0.1Gy, and 1Gy) at 20 min after irradiation. Surprisingly, ChIP-seq data showed an increase in H3K4 trimethylation throughout the genome and this major change in epigenomics was detected even at a low dose radiation of 0.1Gy. We further examined changes in histone modification using a histone mark corresponding to transcriptionally-silent chromatin, H3K9 trimethylation (H3K9me3). We observed a decline in this histone modification after irradiation. Our data show a global change in chromatin structure induced by x-irradiation and such dynamic changes in chromatin conformation may poise cells for phenotypic changes should cells receive appropriate extracellular signals during the recovery period.

In addition to histone modification, chromatin looping is an example of epigenomics because the manner in which chromatin is packaged dictates which genes are to be expressed or not without changing the primary DNA sequences. We have now established a method to study chromatin looping (one specific site interacting with all other sites in the genome mediated by a protein of choice: ChIP-one-to-all) in mammalian cells analyzed by high-throughput sequencing. We will show an example of c-Myc interaction with the rest of the genome in mouse thymocytes. This method will be adopted as a new assay system to examine the effect of low dose radiation on epigenomics (chromatin looping) in the future.