Genome-wide Profiling of Histone H3 Acetylation in MCF-7 Cells in Response to Histone Deacetylase Inhibitors

Seema Verma[1], Julie Stanton[2], John Wyrick[2], Rajiv Prasad[3], Dave Springer[3], John Miller[1]

[1]Washington State University Tri-Cities, Richland, WA
[2]Washington State University, Pullman, WA
[3]Pacific Northwest National Laboratory, Richland WA

Acetylation of lysine residues in core histones is a well characterized reversible posttranslational modification of chromatin that affects gene expression. Histone deacetylation promotes a condensed and transcriptionally repressed chromatin structure. Recent studies (Gui et al. *Proc. Natl. Acad. Sci. USA* 101 (2004) 1241-1246) suggest that histone deacetylase (HDAC) inhibitors de-repress genes involved in cell differentiation, growth arrest, and apoptosis of cancer cells and, because of these features, HDAC inhibitors are currently being used as anti-cancer agents (reviewed in Mai et al. *Med. Res. Rev*. **25** (2005) 261-309). HDAC inhibitors have also been shown to enhance DNA repair (Wojewodzka et al. *Acta Biochim. Pol*. **54** (2007) 63-69), which is believed to be part of the mechanism by which two HDAC inhibitors, valproic acid (VPA) and trichostatin-A (TSA), reduced the lethality of mouse total-body radiation when given either 24 hours before or 1 hour after exposure (Brown et al. *Radiat. Res*. **169** (2009) 474-478).

HDACs have many protein substrates in addition to histones that are involved in gene regulation (reviewed in Xu et al. *Oncogene* **26** (2007) 5541-5542). Consequently, most studies of HDAC inhibitors do not conclusively demonstrate that acetylation of core histones is the root cause of either the anti-tumor or radio-protector effect of HDAC inhibition. To gain more direct evidence of histone modification in the activities of HDAC inhibitors, genome-wide histone H3 acetylation patterns were measured in estrogen receptor positive MCF-7 breast cancer cells treated with 1 mM VPA or 300 nM TSA for 12 hours. Chromatin immunoprecipitation followed by microarray analysis (ChIP-on-chip) was performed using affymetrix human promoter tilling arrays to identify genomic regions associated with acetylation of histone H3 at Lys 9 and Lys 14.

Raw data was analyzed using the MAT algorithm (Johnson et al. *Proc. Natl. Acad. Sci. USA* 103 (2006) 12457-12462) and the affymetrix probes were mapped on the human assembly (hg18) to identify ChIP enriched peaks. Untreated control cells exhibited 1739 chromosome regions of significant antibody binding compared to 16,298 and 5822 significantly acetylated regions in the chromatin of VPA and TSA treated cells, respectively. These results clearly show that the HDAC-inhibitor properties of VPA and TSA lead to extensive H3 acetylation in MCF-7 cells under our exposure conditions. MAT scores were used to compare treated and control cells at common acetylated regions. Common regions of significant antibody binding where the MAT score of treated cells was at least twice as large as control cells were termed “hyperacetylated “ and selected for further analysis of gene function.

Analysis of hyperacetylated ChIP regions among significantly acetylated regions common to treated and control cells using TileMapper revealed 65 and 42 genes whose expression is likely to be up-regulated by VPA and TSA, respectively, due to chromatin remodeling. The MetaCore software (www.genego.com) was used to identify pathways significantly enriched in these genes. The top-ranked network included genes with functions in DNA replication, recombination and repair as well as regulation of cell-cycle, apoptosis and differentiation. Noteworthy among these genes are the following: RAD51C that is required for double-strand break (DSB) repair by homologous recombination, BRIP1 that in a complex with BRCA1 is also involved in DSB repair, WIP1 that inhibits stress induced apoptosis, and BMP7, RhoGEF, and PTP-2 that mediate cell differentiation. These preliminary results suggest that HDAC inhibitors VPA and TSA may induce anti-tumor and radio-protective effects by up-regulating transcriptional targets of p53 that regulate DNA damage response, apoptosis, and cell differentiation.