World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:15, No:12, 2021

DNA Hypomethylating Agents Induced Histone Acetylation Changes in Leukemia

Authors : Sridhar A. Malkaram, Tamer E. Fandy

Abstract : Purpose: 5-Azacytidine (5AC) and decitabine (DC) are DNA hypomethylating agents. We recently demonstrated that both drugs increase the enzymatic activity of the histone deacetylase enzyme SIRT6. Accordingly, we are comparing the changes H3K9 acetylation changes in the whole genome induced by both drugs using leukemia cells. Description of Methods & Materials: Mononuclear cells from the bone marrow of six de-identified naive acute myeloid leukemia (AML) patients were cultured with either 500 nM of DC or 5AC for 72 h followed by ChIP-Seq analysis using a ChIP-validated acetylated-H3K9 (H3K9ac) antibody. Chip-Seq libraries were prepared from treated and untreated cells using SMARTer ThruPLEX DNA- seq kit (Takara Bio, USA) according to the manufacturer's instructions. Libraries were purified and size-selected with AMPure XP beads at 1:1 (v/v) ratio. All libraries were pooled prior to sequencing on an Illumina HiSeq 1500. The dual-indexed single-read Rapid Run was performed with 1x120 cycles at 5 pM final concentration of the library pool. Sequence reads with average Phred quality < 20, with length < 35bp, PCR duplicates, and those aligning to blacklisted regions of the genome were filtered out using Trim Galore v0.4.4 and cutadapt v1.18. Reads were aligned to the reference human genome (hg38) using Bowtie v2.3.4.1 in end-to-end alignment mode. H3K9ac enriched (peak) regions were identified using diffReps v1.55.4 software using input samples for background correction. The statistical significance of differential peak counts was assessed using a negative binomial test using all individuals as replicates. Data & Results: The data from the six patients showed significant (Padj<0.05) acetylation changes at 925 loci after 5AC treatment versus 182 loci after DC treatment. Both drugs induced H3K9 acetylation changes at different chromosomal regions, including promoters, coding exons, introns, and distal intergenic regions. Ten common genes showed H3K9 acetylation changes by both drugs. Approximately 84% of the genes showed an H3K9 acetylation decrease by 5AC versus 54% only by DC. Figures 1 and 2 show the heatmaps for the top 100 genes and the 99 genes showing H3K9 acetylation decrease after 5AC treatment and DC treatment, respectively. Conclusion: Despite the similarity in hypomethylating activity and chemical structure, the effect of both drugs on H3K9 acetylation change was significantly different. More changes in H3K9 acetylation were observed after 5 AC treatments compared to DC. The impact of these changes on gene expression and the clinical efficacy of these drugs requires further investigation.

Keywords : DNA methylation, leukemia, decitabine, 5-Azacytidine, epigenetics
Conference Title : ICPB 2021 : International Conference on Pharmaceutical Biotechnologies
Conference Location : Cairo, Egypt
Conference Dates : December 13-14, 2021