A novel chemical biology strategy to study epigenetic regulation using phenotypic screening

Epigenetic regulation by histone methylation plays a critical role in proper programming of the genome during development. Identifying cellular targets that regulate histone methylation would provide good starting points for drug discovery programs against diseases such as cancer. Here, the authors describe a novel approach that combines phenotypic and target-based drug discovery strategies to study the regulation of Histone 3 K27 tri-methylation (H3K27me3), a chromatin marker in several tumor types.

GSK researchers have built a unique set of 5853 target-annotated chemical probes, called the Biologically Diverse Compound Set (BDCS), which target 736 unique proteins with diverse biological activities. One of the first BDCS applications was in a high-content phenotypic screen to quantify H3K27me3 levels in HCC1806 cells, a breast cancer model system. Increases or decreases in H3K27me3 levels, induced by BDCS compounds, were measured using a highly specific monoclonal antibody against H3K27me3.

After three days treatment with each of the single compounds, cells were imaged using the Opera® High Content Screening System. Figure 1A shows an example of a compound treatment which significantly decreased the H3K27me3 level. Acapella® High Content Imaging and Analysis Software was then used to generate a mask algorithm to identify the entire nuclei region (Figure 1B) and analyze many parameters, including the mean fluorescence intensity of H3K27me3 staining. Hit population analysis and visualization was subsequently performed using TIBCO Spotfire® Software. Figure 2 shows a scatter plot of a multiplexed outcome from the screen. The mean values ± 3x the SD value were used as a cut-off to define hits and divide the compound population into 9 different categories. In total, the screen identified 495 hit compounds that caused a statistically significant change in H3K27me3 level, including 467 H3K27me3 enhancers (Area A) and 28 H3K27me3 suppressors (Area B).

The hit compounds are associated with numerous potential targets. Using bioinformatics analysis, 5 interesting canonical pathways (including ~20 targets) were identified as being likely involved in H3K27me3 regulation in the HCC1806 model system. These new targets, after further validation, could reveal novel therapeutically useful pathways and targets of H3K27me3 regulation. The BDCS could therefore be a powerful drug discovery tool when combined with high content multi-parametric phenotypic screening.

Figure 1 (Left): High content imaging assay to determine the effect of ‘Compound A’ on H3K27me3 levels. (A) HCC1806 cells were incubated with 5 µM Compound A for 3 d and then stained with the DRAQ5 nuclear stain (red) and anti-H3K27me3 (green). Images were acquired using the Opera System. DMSO-treated cells were used as a control. (B) For image analysis, Acapella Software was used to generate a mask algorithm to identify the entire nuclei regions of HCC1806 cells, based on DRAQ5 signals.

Figure 2 (Right): Scatter plot of multiplexed outcomes from the H3K27me3 phenotypic screen. Data was visualized, and hit population analysis performed, using TIBCO Spotfire Software.