Optimizing methodology for the detection of H3K27me3 levels using flow cytometry

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Rationale: We would like to determine H3K27me3 levels in the AML patient cells. Usually this can be performed using the Western blots. However, due to our co-culture system with mouse stromal cells, it can be difficult to interpret the levels of the mark coming solely from the patient cells because the antibody for the mark recognizes both human and mouse H3K27me3. To overcome this, we wanted to see if we could determine H3K27me3 levels using flow cytometry where we can distinguish the human cells using the human CD45 marker.

Methods:

Methodology to fix and stain the cells was similar to the one in the publication from the Hedley lab (Watson, Chow et al. 2014). OCI-AML-20 cells grown in 6-well plates seeded with OP9 stroma were treated for 7 days with either DMSO or EPZ6438 1uM. Media was topped 3-4 days along with addition of compound. After 7 days, cells (~$1x10^6$) were collected into 15mL tubes. The media was removed and cells were fixed using 250uL of 4% paraformaldehyde (PFA) for 10 minutes at room temperature. Cells were washed with PBS and spun down at 1500rpm for 5 minutes. PFA and PBS were removed and cells were stored in PBS at 4 degrees Celsius until ready to stain.

Cells were permeabilized prior to staining with PBS containing 0.1% of Triton X-100by for 10-15 minutes at 37 degrees Celsius. Cells were washed in PBS and then stained at room temperature with 1:50 H3K27me3- Alexa Fluor 647 (Cell Signalling Technology, C36B11) for 1 hour. In the final ten minutes of the hour incubation, 1:201 CD45-Viogreen (Miltenyi) was added to cells.

Cells were analysed using MACSQuant VYB flow cytometer.

Results:

To test this methodology I used OCI-AML-20 described in previous posts (https://opennotebook.thesgc.org/report-culturing-patient-leukemic-cells/ https://opennotebook.thesgc.org/determining-cd34-expression-in-the-presence-and-absence-of-stroma/ https://opennotebook.thesgc.org/determining-the-plasticity-of-cd34-expression/) with and without the EZH2 inhibitor EPZ6438 (Figure 1 and 2).

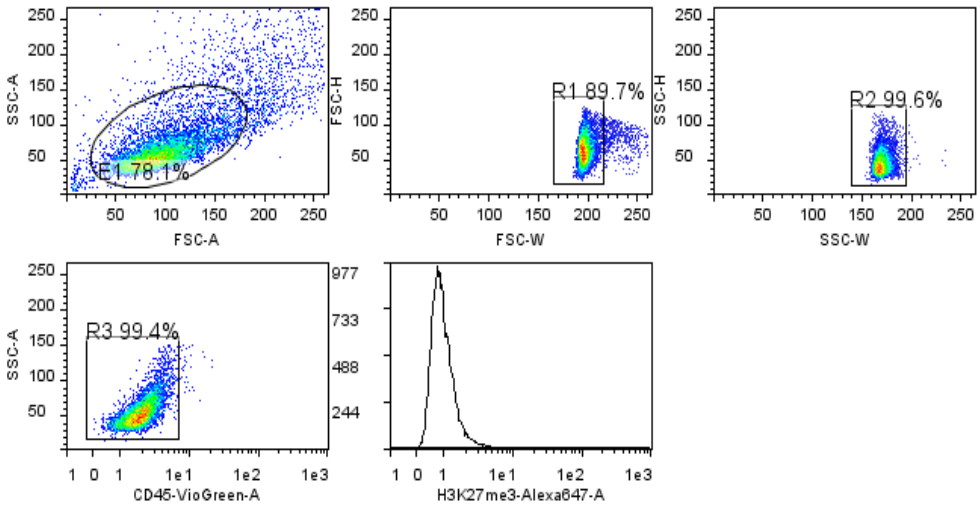

Figure 1: Gating strategy for detection of H3K27me3 levels in OCI-AML-20 cells. Selected cells (SSC vs FSC) were subsequently gated for single cells (H vs W of both SSC and FSC). CD45 positive cells (hematopoietic cells) single cells were analyzed for H3K27me3 fluorescence intensity (Alexa Fluor 647).

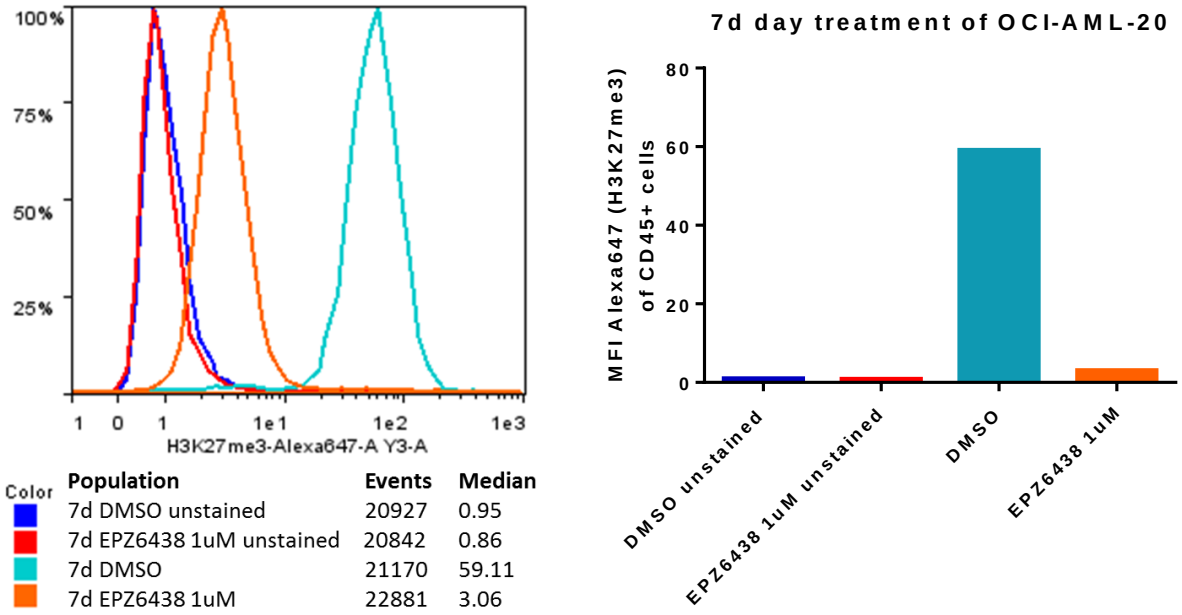

Figure 2: Histogram of H3K27me3 levels in CD45 positive OCI-AML-20 with (EPZ6438; red and orange) and without (DMSO; blue and light blue) 7 days of EZH2 inhibitor treatment. Number of events (cells) is displayed, as well as median fluorescent intensity (MFI) for cell populations. Unstained show almost no fluorescence. DMSO treated cells show a substantial fluorescence signal that is greatly reduced in the presence of EZH2 inhibitor. Results are summarized in the bar graph.

Conclusions:

We were able to determine levels of H3K27me3 in leukemia cells using flow cytometry. We can also detect a significant 19-fold difference in levels of H3K27me3 between control and EZH2 inhibitor treated cells demonstrating the specificity of the H3K27me3 signal in this method (Figure 2). This method for determining H3K27me3 levels will be ideal for use in our patient cells grown in co-culture and for the samples with cell numbers too small for performing western blots.

References

Watson, M., S. Chow, D. Barsyte, C. Arrowsmith, T. V. Shankey, M. Minden and D. Hedley (2014). "The study of epigenetic mechanisms based on the analysis of histone modification patterns by flow cytometry." Cytometry A **85**(1): 78-87.