Finding an antibody to detect EZH1 protein expression – Part 3

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Rationale: We have a good antibody to detect EZH2 levels in our samples. However, for some samples that we see detectable levels of H3K27me3 but no EZH2 we suspect that EZH1 might be substituting for EZH2 in the PRC2 complex. We would like to get a good EZH1 antibody to determine expression levels of this protein in our samples.

Methods:

Cell line lysates were obtained from Magdalena Szewczyk (Research Associate). Westerns were run as previously described (https://zenodo.org/record/1322109#.W6q-mXtKjIU). Blots were blocked for ~30 minutes in blocking buffer (5% BSA with 0.02% sodium azide in 0.1% Tween 20/TBS or PBS) and then incubated overnight at 4 °C with antibodies for EZH1 (rabbit, 1:1000, Cell Signaling Technology, #42088), EZH2 (rabbit, 1:1000, Cell Signaling Technology, #5246) and actin (mouse, 1:3000, Abcam #3280).

For knock downs, Evelyne Lima-Fernandes (Postdoctoral Fellow) had virus prepared and stored at -80 °C for EZH1 and control (luciferase; Luc2). The short hairpin RNAs (shRNAs) A7 and A9 were previously shown to efficiently knock down EZH1 using qRT-PCR and I used these to confirm the antibody specificity. The shRNAs (Clone ID) target the following sequences:

Luc2 (TRCN0000072256): ACGCTGAGTACTTCGAAATGT

EZH1 A7 (TRCN0000002439): GCTACTCGGAAAGGAAACAAA

EZH1 A9 (TRCN0000002441): CCGCCGTGGTTTGTATTCATT

HEK293 cells at 50 percent confluence in 10cm plates were treated with 8µg/mL of hexadimethrine bromide (polybrene) and 500µL unconcentrated virus, along with no virus control. Cells were incubated for 24 hours. After incubation, virus was removed and fresh DMEM media (Wisent) with 10 percent FBS (Wisent) and 100U/100ug/mL penicillin/streptomycin (Gibco) was added. Cells were allowed to recover for 24 hours, and then 1ug/mL of puromycin was added to select transduced cells. This concentration ended up being too low to kill the non-infected controls after three days. Therefore, cells were trypsinized and replated 1:4 with 2ug/mL of puromycin. After two days, media was changed with 1ug/mL puromycin. After an additional two days, puromycin selection was removed and selected cells were allowed to grow for lysate collection. Cells treated with shRNA A7 grew much slower and required an additional two days of growth to collect lysate. Cells were collected and westerns run as previously described (https://zenodo.org/record/1322109#.W6q-mXtKjIU).

Results:

The product monograph for Cell Signaling Technology (CST) EZH1 antibody had a few cell lines that have EZH1 expression (K562, 293 and THP-1). In addition, differentiated C2C12 cells were included based on a previous publication showing EZH1 expression (Bodega, Marasca et al. 2017). I obtained these lysates for these lines from Magdalena's previous experiments and ran the maximum amount I could. I was able to detect a band of the correct size (~85kDa) in only 293 cells (Figure 1). I did not see a band corresponding to EZH1 in K562, THP-1 or C2C12 cells (Figure 1). CST indicates that the antibody is

species specific to human and monkey EZH1 and would possibly explain why I do not see a band in C2C12 which are mouse in origin. Possible protein degradation might explain why we do not see a band for K562 or THP-1.

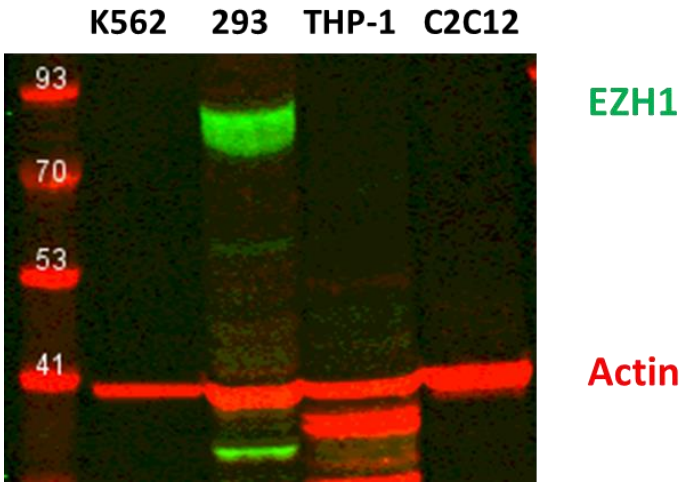

Figure 1: Western blots for EZH1 using cell lines that show EZH1 expression in the CST product monograph and differentiated C2C12 (Bodega, Marasca et al. 2017). We detect a band of the expected size(~85kDa) in the HEK293 lysate.

To test the specificity of the detected EZH1 band, I performed knockdowns using shRNA that were previously shown to knockdown EZH1 using qRT-PCR or control (luciferase) in HEK293 cells. The knockdown to EZH1 (A7 or A9) showed a marked decrease in the band that corresponded to EZH1 in size compared to control (Figure 2A), but no decrease in expression of the related EZH2 protein for A9 shRNA (Figure 2B). This suggests that the EZH1 antibody is recognizing the protein of interest and is specific to EZH1. The A7 shRNA might have some off target affect against EZH2 as we do see some decrease in the expression of EZH2, in addition to EZH1 (Figure 2) and this is likely reflected by the A7 knockdown cells proliferating much slower than control or A9 infected cells.

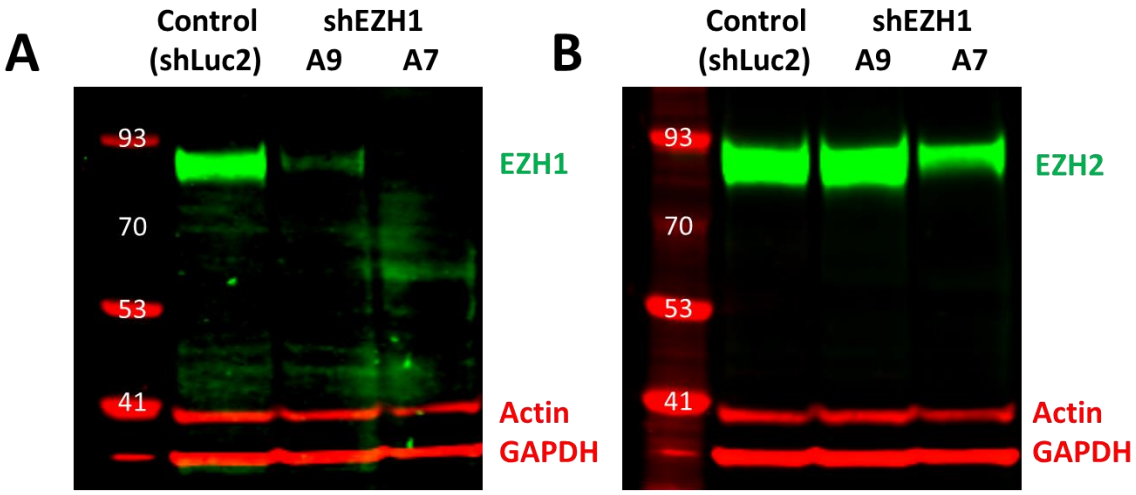

Figure 2: Western blots for (A) EZH1 and (B) EZH2 in HEK293 cells with control (shLuc2) or EZH1 (shEZH1 A9 or A7) knockdown.

Conclusions:

We were able to detect a specific EZH1 band of the correct molecular weight in the HEK293 lysate. I will now test the antibody to determine if we can detect an EZH1 band of the correct size in AML patient cells.

References:

Bodega, B., F. Marasca, V. Ranzani, A. Cherubini, F. Della Valle, M. V. Neguembor, M. Wassef, A. Zippo, C. Lanzuolo, M. Pagani and V. Orlando (2017). "A cytosolic Ezh1 isoform modulates a PRC2-Ezh1 epigenetic adaptive response in postmitotic cells." Nat Struct Mol Biol **24**(5): 444-452.