Finding an antibody to detect EZH1 protein expression – Part 1

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Rationale: We have a good antibody to detect EZH2 levels in patient AML samples. However, for some samples that we see detectable levels of H3K27me3 mark but no EZH2 expression, 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 patient samples.

Methods:

Mouse lysate was obtained from Evelyne Lima Fernandes (postdoctoral fellow). Cell lines and patient cells were lysed at room temperature in buffer containing 50 mM Tris pH 7.6, 400 mM NaCl, 1 mM EDTA, 1% Triton X-100 (v/v), 0.1% SDS (v/v) and cOmplete EDTA-free protease inhibitor cocktail (Roche). Samples were sonicated (QSonica Q800R) at 4°C for a total of 2 minutes with 15 second pulses in 30 second intervals. Protein estimation was performed using Pierce BCA protein assay (Thermo Fisher Scientific) and indicated protein concentration were run with NuPAGE LDS sample buffer (Invitrogen) in 4–12% Bis-Tris protein gels (Invitrogen) with MOPS buffer. Gels were transferred for 1.5 h (80 V) onto PVDF membrane (Millipore) in Tris-glycine transfer buffer containing 20% methanol and 0.05% SDS. Blots were blocked for ~30 minutes in blocking buffer (5% BSA with 0.02% sodium azide in 0.1% Tween 20/PBS) and then incubated overnight at 4°C with antibodies for EZH1 (rabbit, 1:250, Abcam ab64850; rabbit, 1:500 Active Motif) or ENX-2/EZH1 (mouse, 1:100, Santa Cruz) and actin (mouse, 1:3000, Abcam #3280). The next day, the blots were washed three times with 0.1% Tween 20/PBS, and then incubated with goat anti-rabbit IgG (IRDye 800-conjugated, LiCor #926-32211) and donkey anti-mouse IgG (IRDye 680-conjugated, LiCor #926-68072) antibodies (1:5000) in Odyssey Blocking Buffer (LiCor) for 1 hour at room temperature. Blots were again washed three times with 0.1% Tween 20/PBS and the signal was read on an Odyssey scanner (LiCor) at 800 and 700 nm.

Results:

The EZH1 antibodies were tested using mouse brain lysate where the protein is expected to be expressed (http://www.informatics.jax.org/marker/MGI:1097695). EZH1 has an expected size of ~85kDa. No band was detected for either the Abcam or the Santa Cruz antibodies (Figure 1A). A faint band of the expected size was seen using the Active Motif antibody (Figure 1B). I then tested if we could see a similar band in an AML cell line (USCD-AML1) and patient cells that we expect to have EZH1 expression (151077, previously saw no EZH2 expression but can detect H3K27me3 mark).

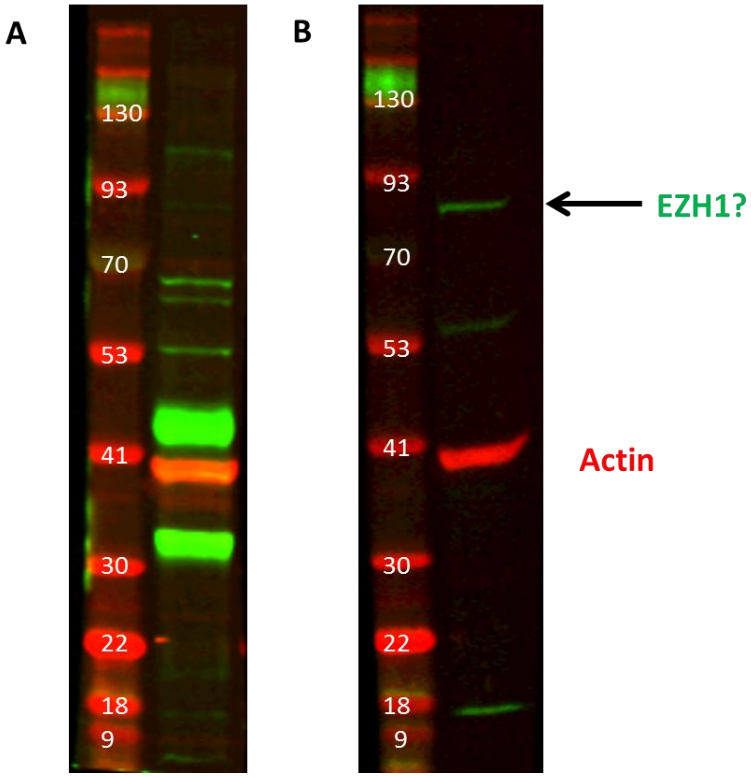

Figure 1: Western blots probing 50ug of mouse brain lysate with antibodies to actin (red) and either (A) Abcam (ab64850; rabbit) and Santa Cruz (mouse) or (B) Active Motif EZH1 (rabbit). A band at the approximate size expected for EZH1 (~85kDa) was seen in (B) (green).

We were not able to detect any band corresponding to EZH1 using the Active Motif antibody in either cell line or patient lysate (Figure 2).

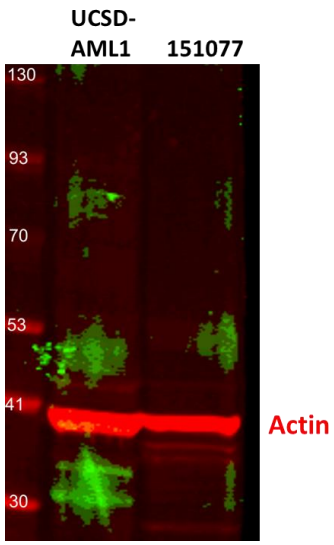

Figure 2: Western blot of USCD-AML1 (75ug protein) and patient cells 151077 (50ug protein) with Active Motif EZH1 antibody does not detect a band.

Conclusions:

I was not able to detect EZH1 with antibodies that we had from previous experiments and will order additional antibodies to determine EZH1 expression in AML patient cells. Unfortunately, the antibody that has been used in multiple publications is no longer available in Abcam's catalog (https://www.citeab.com/antibodies/729109-ab13665-anti-ezh1-antibody). A different antibody from Abcam has been used in one publication is ab176115 (Li, Hart et al. 2013). The authors used the antibody in mouse and human neuronal cells. Another antibody we will obtain is from Novus (NB100-56358SS). The product webpage had a positive review showing a good band on a Western, and lists that it has also been used in a publication (Grimaldi, Christian et al. 2011).

References:

Grimaldi, G., M. Christian, J. H. Steel, P. Henriet, M. Poutanen and J. J. Brosens (2011). "Down-regulation of the histone methyltransferase EZH2 contributes to the epigenetic programming of decidualizing human endometrial stromal cells." Mol Endocrinol **25**(11): 1892-1903.

Li, J., R. P. Hart, E. M. Mallimo, M. R. Swerdel, A. W. Kusnecov and K. Herrup (2013). "EZH2-mediated H3K27 trimethylation mediates neurodegeneration in ataxia-telangiectasia." Nat Neurosci **16**(12): 1745-1753.