CRISPR knockout of EZH1 in AML cell line

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Rationale: We wanted to ensure the specificity of the EZH1 antibody that I was working with in previous posts, as well as creating a useful reagent to use in future experiments by generating a CRISPR knockout line of EZH1.

Methods:

Stable Cas9-expressing OCI-AML-2 or OCI-AML-3 cell line were generated using the pCDH- EF1-Cas9(NLS)-T2A-copGFP plasmid (Cellecta, USA), which was kindly provided by Steven Chan from University Health Network. In brief, Cas9(NLS)-T2A-copGFP lentiviral particles were generated in HEK293FT (Invitrogen, Canada) cells, and OCI-AML-2 or 3 cells were transduced for 24h to 48h. GFP-positive cells were sorted by FACS to generate a purified Cas9(NLS)-T2A-copGFP population.

sgRNAs targeting EZH1 were designed using an online tool (http://guides.sanjanalab.org/#/), while the LacZ negative control sequence has been previously described (Hart, Chandrashekhar et al. 2015). Sequences were subsequently cloned into the lentiGuide-Puro plasmid (Addgene 52963), which has been previously described (Sanjana, Shalem et al. 2014).

sgEZH1-1	CCCACCTCAACTCTGCGTAG
sgEZH1-2	AGCGCCGGCAAAAAAGTGTG
sgEZH1-3	TTGGTAGTTGTACACTTGTG
sgLacZ	CCCGAATCTCTATCGTGCGG

OCI-AML-3 Cas9 expressing cells were infected with sgRNA lentivirus similar to what I described previously with OP9 cells but in 6 well format (https://zenodo.org/record/1154257#.XNRKxzBKjIU). Infected cells were puromycin selected (3ug/mL) for 3-4 days and then allowed to recover before determining protein levels.

After ~3 weeks of culturing the cells, 2 million cells were collected and lysates were prepared and westerns were run as previously described (https://zenodo.org/record/1322109#.W-yYMDhKjIU) using antibodies from previous experiment (https://zenodo.org/record/1435780#.W-yYhThKjIU).

Results:

In my previous post I was able to detect a likely specific EZH1 band in HEK293 cells using EZH1 knockdown (https://zenodo.org/record/1435780#.W-yYhThKjIU). To determine if this band was specific we wanted to generate CRISPR knockout lines of EZH1. We obtained Cas9 expressing AML cell lines from the He lab and determined if either OCI-AML-2 or OCI-AML-3 had detectable levels of both EZH1 and EZH2 (Figure 1). We found that OCI-AML-3 expressed both proteins and could be used to preform knockout lines.

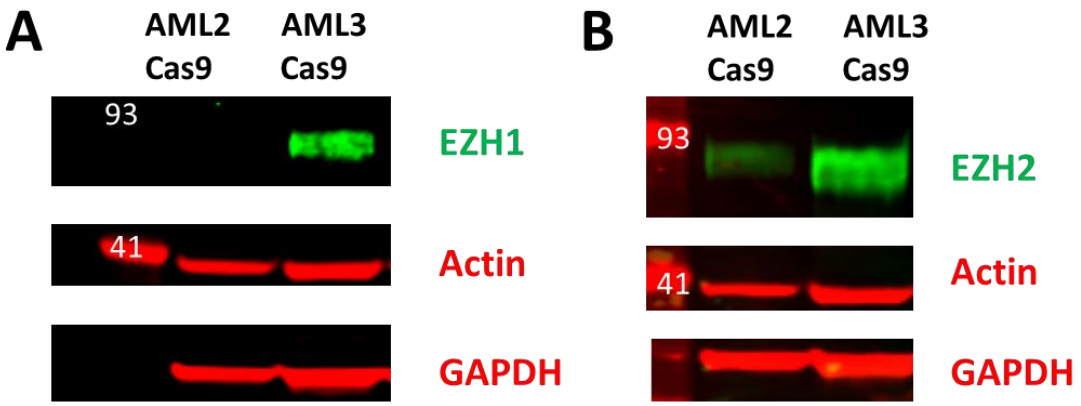

Figure 1: Westerns of Cas9 expressing AML cell lines OCI-AML-2 (AML2) and OCI-AML-3 (AML3) to determine expression levels of (A) EZH1 or (B) EZH2 with Actin and GAPDH loading controls.

After CRISPR knockout was performed, I collected cells from either control guide (sgLacZ) or either of the EZH1 guide RNAs. One of the guide RNAs for EZH1 (sg1) had poor growth after the culture period and I could not collect enough cells to determine protein levels. For EZH1 knockout guides 2 and 3, I saw no detectable levels of the EZH1 protein compared to control (Figure 2A). In contrast, the knockouts had no effect on EZH2 protein levels (Figure 2B).

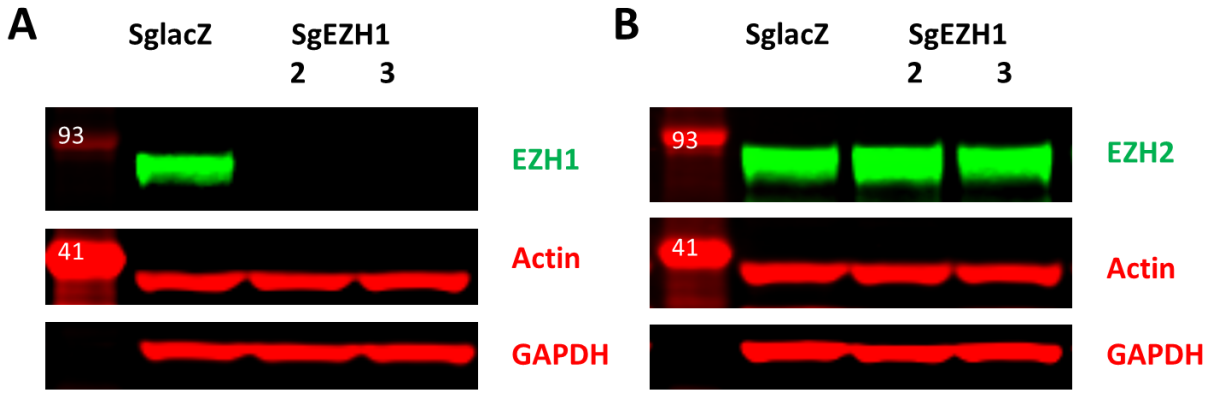

Figure 2: Westerns of Crispr knockouts in OCI-AML-3 Cas9 cells with either control guide RNA (sgLacZ) or guide RNA to EZH1. Levels of EZH1 were undetectable with knockout (A) and did not affect EZH2 levels (B).

Conclusions:

We have a specific antibody to EZH1 that can be used to detect EZH1 expression levels in patient cells. We also generated an AML cell line with EZH1 knockout that could be a useful tool for further experiments.

References:

Hart, T., M. Chandrashekhar, M. Aregger, Z. Steinhart, K. R. Brown, G. MacLeod, M. Mis, M. Zimmermann, A. Fradet-Turcotte, S. Sun, P. Mero, P. Dirks, S. Sidhu, F. P. Roth, O. S. Rissland, D. Durocher, S. Angers and J. Moffat (2015). "High-Resolution CRISPR Screens Reveal Fitness Genes and Genotype-Specific Cancer Liabilities." Cell **163**(6): 1515-1526.

Sanjana, N. E., O. Shalem and F. Zhang (2014). "Improved vectors and genome-wide libraries for CRISPR screening." Nat Methods **11**(8): 783-784.