

Finding an antibody to detect EZH1 protein expression – Part 4

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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:

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 specific EZH1 band in HEK293 cells (<https://zenodo.org/record/1435780#.W-yYhThKjIU>) and now I wanted to determine if the same antibody could detect EZH1 in lysate from patient cells. I ran another western with lysate from HEK293 cells as a positive control, along with patient cells (151077 and 150860) and the cell line I have previously described (OCI-AML-20; <https://zenodo.org/record/1186848#.W-yZzThKjIU>). I discussed in a previous post the use of 151077 and OCI-AML-20 (<https://zenodo.org/record/1326848#.W-yYhDhKjIU>). In contrast, we think 150860 would express EZH2 and likely not have EZH1 expression. We are able to detect EZH1 expression in all the samples, except 150860 (Figure 1). As expected, we see an intense band in 151077 and a weaker signal in OCI-AML-20 (Figure 1).

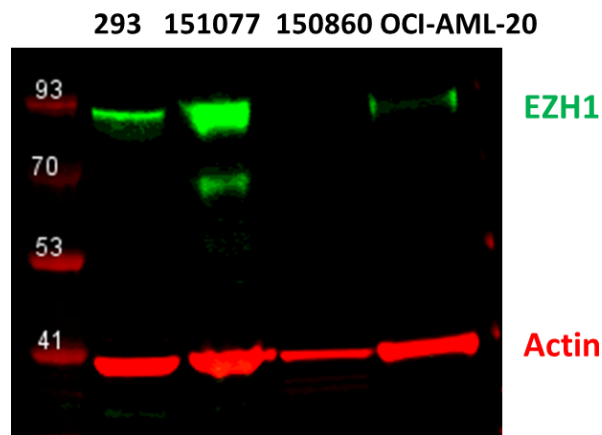


Figure 1: Western blot for EZH1 (green) and actin (red) with ~50ug protein loaded. A band of expected size (~85kDa) is seen in all samples except 150860.

Conclusions:

We have a specific antibody to EZH1 that can be used to detect EZH1 expression levels in patient cells. We can now use this antibody to detect EZH1 expression in additional patient samples.