Western Bloting NSD3 in AML Cell Lines

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Objective. To test an NSD3 antibody from AbCam ([EPR13813] - N-terminal (ab180500)) and assay NSD3 protein expression levels in AML cell lines shown to require NSD3.

Rationale. In order to study NSD3 in the context of AML, I first need to generate and evaluate the best reagents to move forward with for future experiments. This includes characterizing model cell lines as well as antibodies that can be used for future experiments.

Experimental Details:

1. Culture of Cell Lines

Cells were culturated in suspension at 37^{o}C following standard protocols. The cells were maintained at 0.5-2 x 10^6 cells / ml. Both HL-60 and MOLM-13 cells have been shown to require NSD3 for proliferation (Mol Cell. 2015 Dec 17; 60(6): 847–859. PMCID: PMC4688131)

Cell Lines:

Name	Growth Media	Notes
MOLM-13	IMDM +10% FBS & Pen/Strep	MLL-AF9 Fusion (DSMZ no: ACC554)
UCSD-AML1(cherry)	IMDM +GMCSF +10% FBS & Pen/Strep	EVI1 Overexpression (DSMZ no: ACC 691)
HL-60	RPMI +10%FBS & Pen/Strep	acute promyelocytic leukemia (ATCC - CCL-240)

2. SDS-PAGE & Western Blot Analysis of Whole Cell Lysates

Lysis Buffer:
* 20 mM Tris-HCl pH8
* 150 mM NaCl
* 10 mM MgCl2
* 1mM EDTA
* 0.5 % Triton X-100

Add fresh protease Inhibitors (100x) & benzoase (10 000x) prior to lysis.

1. 2 ml of cells (~2 x 10^6) were pelleted by centrifugation at 300 x G for 5 min.

2. The supernatent was removed and cells were washed 1x in 500 μL 1 x buffer, then resuspended in 100 μL of lysis buffer.

3. Lysates were incubated on ice for 10 min at RT , then SDS added to 1% (final concentration).

4. BCA protein concentration estimation was performed using Pierce BCA Protein Assay Kit (Cat# 23225).

5. 50 μg of total protein was run on a NuPAGE 4-12% Bis-Tris Protein Gel (NP0322BOX) in 1x MOPS

Running Buffer at 100 volts.

6. Proteins transfered overnight at 30 volts in 1 x tris-glycine transfer buffer to a 0.2 μm PVDF membrane.

7. Membrane blocked in 5% milk in PBS-T (1x PBS - 0.1% Tween-20) for 30 min at RT.

8. Membrane cut and probed with antibodies diluted in 5% BSA in PBS-T for 1 hour at RT. Dilutions shown below:

Target	Supplier - Cat#	Dilution
NSD3 (RabMono)	AbCam - ab180500	1:5000
Actin (MouseMono)	AbCam - ab3280	1:2000
Histone-H3 (RabPoly)	AbCam - ab70550	1:5000

9. Membranes washed 3x - 10min in PBS-T.

10. Incubated with secondary LiCor antibodies to mouse and rabbit in Licor Odyssey Blocking buffer (927-40000) diluted 1 in 5.

11. Membranes washed 3x - 10 min PBS-T and one additional wash in 1 x PBS for 5 min.

12. Blots were imaged on a Licor Odyssey CLx Imaging System.

13. Annotated results shown below (Figure 1)

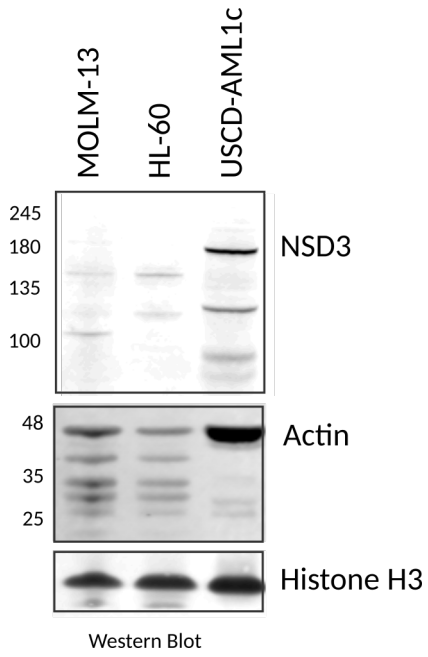

Figure 1: Western Blot of AML Cell Lines

3. Result

There was no NSD3 signal at correct molecular weight for short or long isoforms from MOLM-13 and HL-60 cell lysates, however based on actin staining it appears that there may be some degradation in these samples.

UCSD-AML1 cells show a signal at the appropriate molecular weight for the long isoform. Will need to rerun with fresh lysate. Also, it may be worth trying to resuspend samples directly in SDS loading buffer to avoid any potential protein degradation after cell lysis.

These samples were also run on a SDS-PAGE gel stained with coomassie to check protein integrity (Figure 2) Again, with HL-60 and MOLM-13 cells I observed fewer high molecular weight proteins suggesting possible protein degradation in these cell lines.

Figure 2 Loading

Lane	Sample
1.	Ladder
2.	MOLM-13
3.	UCSD-AML1c
4.	HL-60

Ladder = BLUeye Prestained (FroggaBio Cat# PM007)

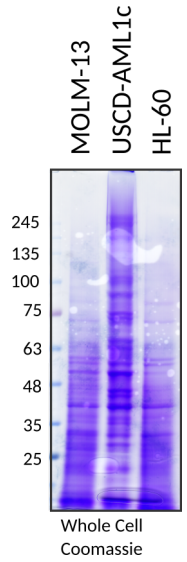

Figure 2: Coomassie stain of whole cell extract from AML cell lines.

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