Measuring E-cadherin Expression in NSD3short Overexpressing H1299 Cells

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Objective. During epithelial to mesenchymal transitioning (EMT), transcription networks become activated and cytoskeletal rearrangements take place. These events promote migratory capacity and invasiveness. Cadherins are a family of cell adhesion molecules central in transitioning to a more mesenchymal state. E-cadherin, a marker for polarized epithelial cells, is downregulated during EMT. Here, I investigate the effect of NSD3short overexpression on E-cadherin expression in H1299 cells, which would support NSD3 as a EMT driver.

1. Experimental Details

1.1 Cell Lysis & Western Blotting

H1299 cells stably overexpressing NSD3short-3xFLAG (wild-type or W284A) were lysed and western blotting performed as follows;

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) & benzonase (10 000x) prior to lysis.

1. 60 uL of lysis buffer with fresh protease inhibitors and benzonase was added directly to each well of the plate.
2. Lysates were incubated on ice for 5 min at RT , then SDS added to 1% (final concentration).
3. Lysates were collected into 1.5 ml eppendorf tubes.
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 transferred at 1.5 hrs at 80 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 overnight at 4^{o}C. Dilutions shown below:

Target	Supplier - Cat#	Dilution
NSD3 (RabMono)	AbCam - ab180500	1:5000
E-cadherin (RabPoly)	abcam - ab53033	1:1000
aTubulin (MouseMon)	Santa Cruz - sc-8035	1:5000
Histone H3 (RabPoly)	abcam - ab70550	1:2500

9. Membranes washed 3x - ~10 min in PBS-T.
10. Incubated with secondary LiCor antibodies to mouse and rabbit (diluted - 1:5000) 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. Annotated results shown below (Figure 1).

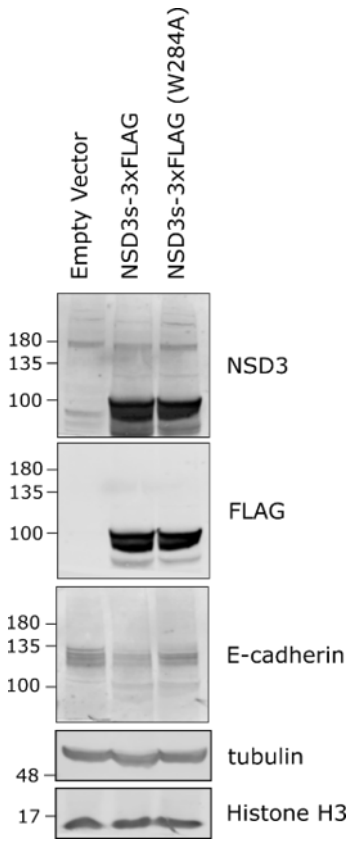

Figure 1: Western blot of H1299 Cells Overexpressing NSD3short

2. Results & Obeservations

Observations. In stable cells overexpressing either wild-type NSD3short or a W284A PWWP1 mutant, E-cadherin protein levels appear reduced relative to an empty vector control cell line. This result supports further investigation of NSD3 as a potentiator of EMT in lung cancer cells.

Next, I will regenerate stable cell lines by lentiviral transduction and selection to confirm this observation as well as testing in NSD3 knockdown conditions.

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