

NSD3Short Represses E-cadherin Expression in A549 Lung Cancer Cells

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Objective

In a previous experiment (exp023), we show that siRNA-mediated knockdown of NSD3 increases expression of E-cadherin, a marker of epithelial cell identity. To determine which isoform of NSD3 is involved in promoting epithelial to mesenchymal transition, I have designed siRNA that targets either the long or short isoform and again use E-cadherin expression as a marker. I have also started using A549 lung epithelial cancer cells (<https://www.atcc.org/Products/All/CCL-185>) as my primary model. This cell line proliferates faster and is more amenable to in vitro culture conditions than H1299 cells I was using previously.

Experimental Details

Transfection of A549 cells with NSD3-targeting and control siRNA was performed in duplicate following the Life Technologies protocol for RNAiMAX reagent (https://tools.thermofisher.com/content/sfs/manuals/Lipofectamine_RNAiMAX_Reag_protocol.pdf). Approximately 72 hours post-transfection, cells were lysed and western blotted. A detailed protocol for cell lysis and western blotting can be found on protocol.io (<https://dx.doi.org/10.17504/protocols.io.pxndpme>).

siRNA Used

Target	Supplier - Cat#	Final Concentration
Non-targeting Control	Sigma - SIC001	5 nM
NSD3	Ambion - 4392420-s29725	5 nM
NSD3Long	IDT - CD.Ri.108909.13.1	5 nM
NSD3Short	IDT - CD.Ri.108912.13.4	5 nM

Antibodies Used

Target	Supplier - Cat#	Dilution
NSD3 (RabPoly)	ProteinTech - 11345-1-AP	1:1000
E-cadherin (RabMono)	abcam - ab40772	1:10 000
Actin (MouseMon)	abcam - ab3280	1:5000

Observations

Here we observe that NSD3 knockdown in A549 cells results in increased E-cadherin expression, as shown previously with H1299 cells. This phenotype is associated exclusively with knockdown of the short isoform, which lacks the SET H3K36me2 methyltransferase domain, implying that the catalytic activity of this enzyme is not required for its role in EMT.

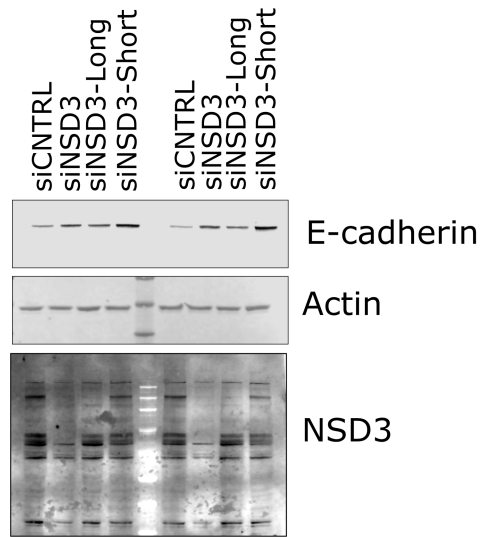


Figure 1: E-cadherin Expression in Response to NSD3 Knockdown

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