Understanding the putative role of SIRPα as a binding ligand for Laminin

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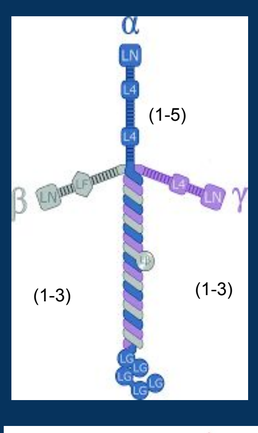
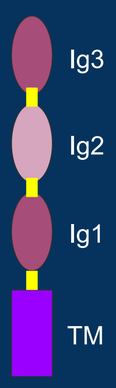
Background:

- Neuronal synapses are the loci of information transfer between two neurons. In order to form synapses, the presynaptic and postsynaptic neurons need to spatially stabilise axonal and dendritic processes. Dendritic spine stability is achieved by homophilic or protein-ECM interaction of transmembrane molecules like Neuroligins. Lack of synaptic stability is the cellular hallmark of conditions characterised by learning impairment such as autism.
- Signal regulatory protein α (SIRPα or SHPS1) belongs to the immunoglobulin superfamily of proteins and has three immunoglobulin like domains (Ig), one transmembrane domain and downstream phosphatase activity. It's role as a ligand for CD47 in immune cells like macrophages has been well documented but its function in neurons is still unknown.
- Laminin is a fibrous protein that is replete in the ECM that binds cell adhesion molecules like integrins and is essential for normal neurodevelopment

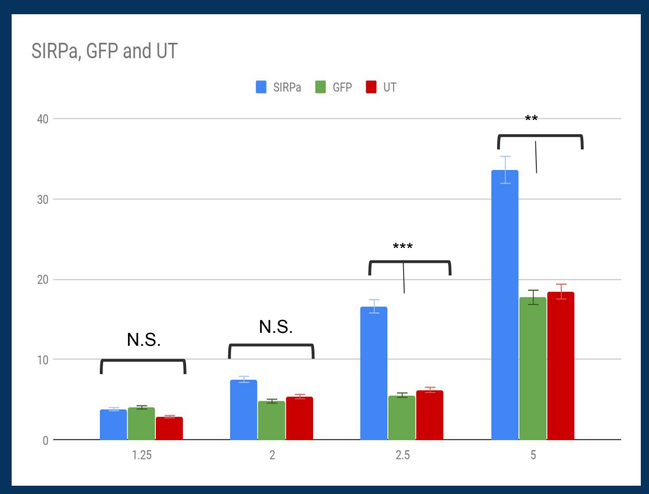




- Laminin knockouts in brain slices have shown reduced expression of SIRPα, indicating a putative interaction between the two molecules. **In this study, we determine whether SIRPα binds particular Laminin substrates and aids synapse stability.**

1. SIRPα Transfected Cells Preferentially Adhere to Laminin 521

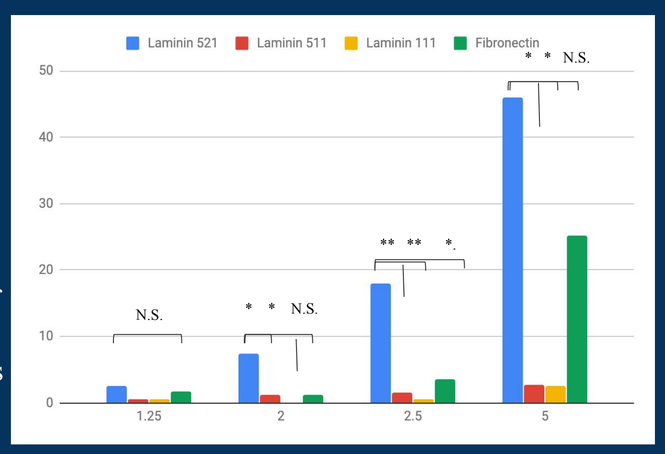
In the standard binding assay, stopped at varied times, cells transfected for 24 hours with SIRPα show greater affinity in adhering to Laminin 521 coated plates at concentrations greater than 2.5 µg/ml. Readings measured fluorescence due to cell binding and were normalised for initial cell population per well. Each "n" is an average of three wells per run



$p < 0.05$ * ; 0.01 ** ; 0.001 ***

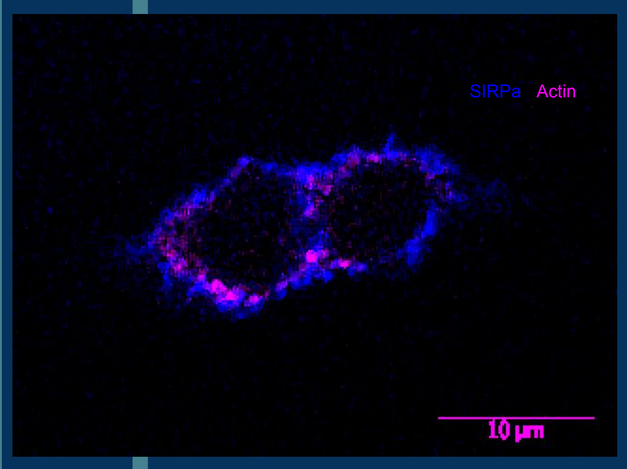
2. Adherence is restricted to Laminin 521 as a substrate

The assay was repeated with different ECM component proteins to assess specificity of adherence for Laminin. Different isoforms of the Laminin chains were used to determine specificity of binding to specific subtypes. Each sample has an "n" of 3 averaged across 3 wells.

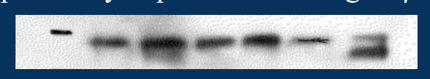


From the results above, it is clear that adherence specificity is for Laminin 521, indicating that SIRPα may bind to the β2 chain of Laminin. Human laminin β2 deficiency has been shown to cause developmental defects such as mesangial sclerosis and eye abnormalities (Zenker, 2004)

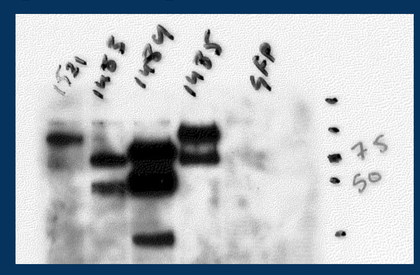
3. Quality control; ensuring experimental and ruling out external causes of positive result



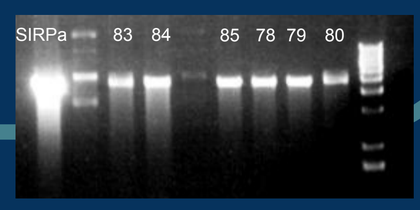
- Western to confirm there is no compensatory expression of integrin β2



- Western Blot to confirm expected expression of protein

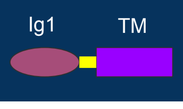


- Gel Electrophoresis to confirm integrity of DNA constructs

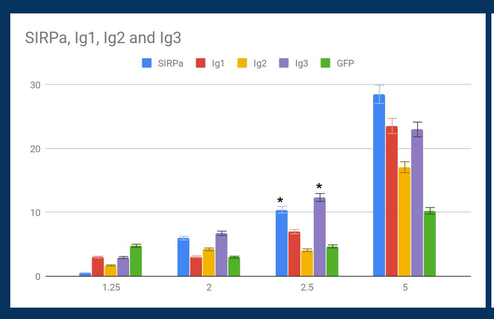


4. Ig3 expression is sufficient for cell adhesion to Laminin 521

Sufficiency:



Necessity:



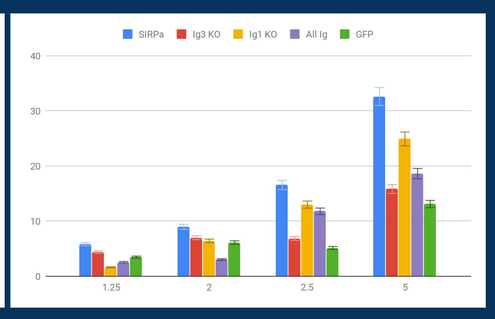


Fig 1: Individual Ig domain expression tested for significance against binding of GFP showing Ig3 is sufficient for adhesion phenotype;
Fig 2: Ig domain knockouts, not statistically significant at $p<0.05$ which might be as n is not high enough or the knockout affects protein integrity

Summary:

- HEK cells, with no endogenous SIRPα expression, when transfected with human SIRPα display adherence to Laminin α5β2γ1.
- The phenotype is restricted to only wells coated with Laminin 521, indicating the β2 chain plays a role in the observed phenotype
- The expression of only the Ig3 domain of the protein is sufficient to recapitulate this phenotype, indicating the laminin binding site may be located on the Ig3 domain of SIRPα

Future Steps:

- Purify Laminin β2 and narrow down on the amino acid binding sites of SIRPα. Check the phenotype of β2 knock-downs and SIRPα.knockdown in neuronal cell culture and try rescuing the phenotype
- Try to resolve the signalling cascade that might be downstream of SIRPα.
- Expression of SIRPα mutations in animal models to assess effect on development

Citations:

1. Umemori, Hisashi, and Joshua R. Sanes. "Signal Regulatory Proteins (SIRPS) Are Secreted Presynaptic Organizing Molecules." *Journal of Biological Chemistry*, vol. 283, no. 49, 2008, pp. 34053–34061., doi:10.1074/jbc.m805729200.
2. Zenker, Martin, et al. "Human Laminin β2 Deficiency Causes Congenital Nephrosis with Mesangial Sclerosis and Distinct Eye Abnormalities." *Human Molecular Genetics*, vol. 13, no. 21, 2004, pp. 2625–2632., doi:10.1093/hmg/ddh284.
3. Südhof, Thomas C. "Towards an Understanding of Synapse Formation." *Neuron*, vol. 100, no. 2, 2018, pp. 276–293.

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