Role of Laminin211 in Neuregulin induced de-myelination in vitro
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Peripheral nervous system (PNS)
The peripheral nervous system (PNS) consists of all of the nerves and ganglia outside of the central nervous system (CNS) (i.e., the brain and spinal cord).

The PNS regulates sensation, movement, and organ function by carrying information from the CNS out to the various body systems, and relaying information from these systems back to the CNS.

Myelin in PNS
Myelin is a multilaminar structure produced by glial cells around axons. In the peripheral nervous system, Schwann cells (SCs) are the glial cells that myelinate axons.

Factors that induce de-myelination:
1. Leprosy
Chronic disease caused by the bacteria Mycobacterium leprae

Early Stages: M. Leprae invades the PNS utilizing SCs for proliferation and as a consequence induces SC de-differentiation and myelin breakdown.

Lasting effects: Leprosy can be cured, but any damage already done on peripheral nervous system is irreversible.

Symptoms: paralysis, loss of sensation and consequent unintentional mutilation of hands and feet.

2. Injury
Compression or transection of a nerve

Early Stages: Axons can degenerate and SCs de-differentiate and demyelinate.

Lasting effects: Gain of function is never complete due to inability of re-myelination and axonal regeneration is limited.

Symptoms: Loss of sensation, movement and feeling of numbness.

3. Genetic disorders
Charcot-Marie-Tooth Disease: mutations causing nerve atrophy

Early Stages: Production of abnormal myelin because of its protein components causing de-myelination.

Lasting effects: De-myelinating SCs cause abnormal axon structure and function. This may result in axon degeneration or cause axonal malfunction

Symptoms: There is loss of touch sensation in the extremities characteristic in various types of the disease.

Another example is Krabbe disease: affects both in central and peripheral nervous system.

Relevance of the study
Understanding factors that trigger de-myelination may reveal neuroprotective factors important in injury and disease.

Neuregulin 1
Neuregulin 1 is an axonal signal that can affect:

- SCs proliferation and survival
- SCs recognition of larger diameter axons causing them to differentiate into pro-myelinating SCs.
- The initiation of myelination as well as the thickness of myelin that is developed

Can also trigger de-myelination. The reason for this is unknown.

Neuregulin 1 isosforms:
- Neuregulin 1 Receptor is a Dimer

Neuregulin 1 induced de-myelination in vitro

Myelinated Schwann Cell
- Neuregulin 1 (EGF domain) treatment for 2 days

The Leprosy bacillus binds laminin receptors to enter Schwann cells.

Hypothesis
In the absence of Laminin 211, there will be a loss of regulatory inhibition on Neuregulin 1 causing a stronger de-myelination phenotype.

In vitro Experimental Approach
- Isolation of Dorsal Root Ganglion (DRG) cells
- Dissociation of cells
- Neurons and Schwann cells treated with media
- Change in type of media to induce myelination in vitro
- Induce De-myelination with 10M Neuregulin
- 2 – 4 days of Treatment

Quantification of Results
Neuregulin 1 (EGF domain) treatment for 2 days

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MBP (total)</th>
<th>MBP (untreated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10.00</td>
<td>0.00</td>
</tr>
<tr>
<td>dy3k/dy3k</td>
<td>2.46</td>
<td>1.63</td>
</tr>
<tr>
<td>NRG1 2 days</td>
<td>14.20</td>
<td>12.47</td>
</tr>
</tbody>
</table>

Laminin 211 deficient mice explants at 2 days of treatment, indicates:
- The absence of Laminin 1 enhances Neuregulin 1 induced de-myelination (in dy3k/dy3k)
- Heterozygous mice (dy3k+/+) display a stronger phenotype than the complete null.

Neuregulin 1 (EGF domain) treatment for 4 days

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<td>11.07</td>
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Laminin 211 deficient mice explants at 4 days of treatment, indicates:
- Laminin 211 absence enhances Neuregulin 1 induced de-myelination (in dy3k/dy3k)
- Heterozygous mice (dy3k+/+) display a stronger phenotype than the complete null.

Conclusions
- Absence of Laminin 211 causes a stronger de-myelination after Neuregulin 1 (EGF domain) treatment compared to WT.
- Reduction of Laminin 211 is sufficient to increase de-myelination after Neuregulin 1 EGF domain treatment.

These results suggest that Laminin 211 negatively regulates Neuregulin 1 pathways. In fact, when we remove Laminin 211 there is the loss of the inhibition on Neuregulin 1 pathway causing stronger de-myelination.

On Going and Future Work
- Confirm these results.
- Check if different isoforms of Neuregulin 1 have different effect on de-myelination in absence of Laminin 211.
- Analyze the pathways activated downstream of Neuregulin 1 during de-myelination in Laminin 211 deficient cells.

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