Understanding post-transcriptional mechanisms of Neuropathic pain with single molecule detection

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Abstract

Neuropathic pain is a chronic condition which can arise following damage to the somatosensory system. The molecular mechanisms of neuropathic pain remain incompletely understood but require enduring alterations in protein synthesis affecting neuronal signaling and excitability. We investigate the roles of non-coding RNA regulatory pathways in impacting hyperalgesia and determining the mRNA complement recruited during this protein synthesis response in neuropathic pain. Nerve injury alters the expression of many miRNAs, including the highly conserved Let-7 family miRNAs, which repress pro-growth mRNAs and are implicated in axon growth and brain circuit formation. The Lin28 RNA binding protein can prevent maturation of let-7 precursor RNAs; consequently, increased Lin28 signaling promotes pro-growth gene expression. The regulation and potential roles role of Lin28/Let-7 pathway in neuropathic pain remain unexplored. Using complementary mouse models of neuropathic pain, we evaluate molecular mechanisms underlying pain using single molecule detection and genetic manipulation. Sensitive RNA imaging assays, RNAScope in situ hybridization (ISH), amplify single RNA target signals in fixed tissues to allow mapping of the spatiotemporal patterns and cell type specificity of changes in non-coding RNA regulatory pathways. Digital PCR is used to provide sensitive and quantitative validation.

Background

- The molecular mechanism of neuropathic pain involves selective programs of gene expression such as programs support hyper-excitability. (Arkady Khoutorsky et. Al; 2018)
- MAPK pathway activation serves as a key step in generation of neuropathic pain. Injured animal administered an ERK inhibitor lose hypersensitivity to mechanical and heat stimulation. (koichi obata et. Al; 2004, Weiya Ma et. Al; 2005)

Figure 1: Neurotrophic stimulation can rapidly lower let-7 miRNA levels through MAPK mediated TRBP and Lin28a phosphorylation.

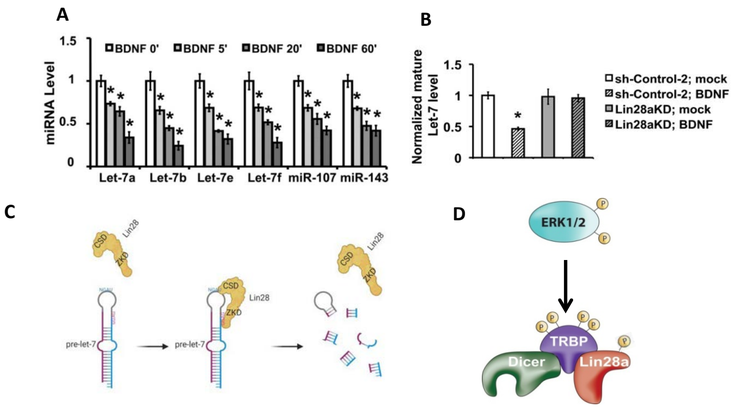
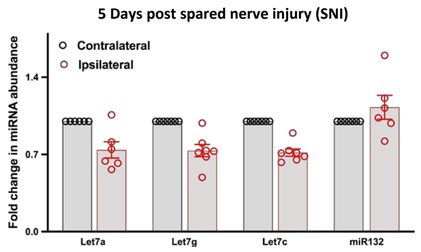


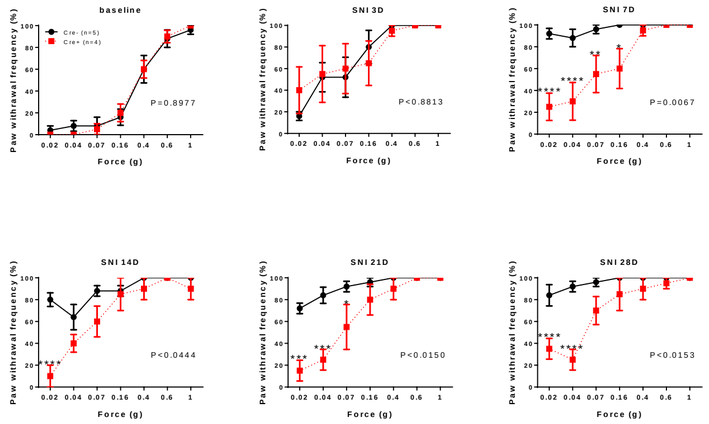
Figure 2: Let-7 growth suppressor miRNAs are down-regulated following nerve injury.



Preliminary data

We use spared nerve injury (SNI) as the neuropathic pain model, which involves a lesion of tibial and peroneal nerves and leaves the remaining sural nerve intact. We acquire the Von Frey assay as our pain behavioral study model to test effect of conditional loss of lin28a function in selective neuron types on hypersensitivity development.

Figure 1: Advilin Cre-ER Lin28aLoxP mice Pre and Post SNI behavioral data in a time course. (**Sangmin Jeon, Michael Caterina Lab**). Lin28a knock out mice show a defect in full hypersensitivity generation.



Methods

Figure 1: RNAScope amplify single molecule signal by sequential hybridization of amplifiers and label probes.

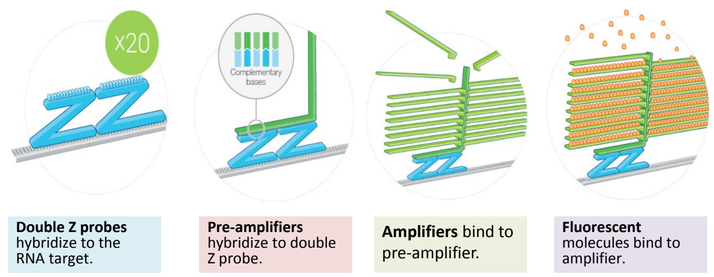
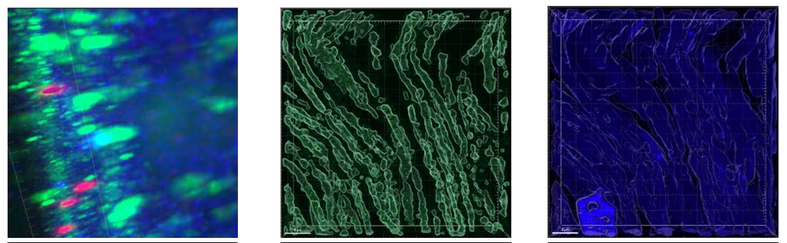


Figure 2: Creating 3D masking surfaces for Lin28 RNAScope puncta, MAP2 neuronal processes, and S100 Schwann cells with Bitplane IMARIS.



Lin28 RNAScope virtual puncta | MAP2 Virtual neuronal processes | S100 Virtual Schwann cells

Results

Figure 1: Injured cells have higher Lin28a mRNA expression than uninjured cells on the ipsilateral side at 3 days post injury (dpi).

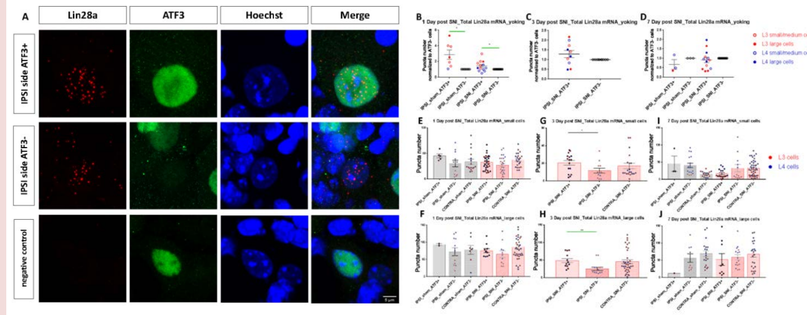


Figure 2: Injured neuronal processes and surrounding Schwann cells have higher detected Lin28a and Lin28b mRNA levels than uninjured neurons on the ipsilateral side at 3 days post injury (dpi).

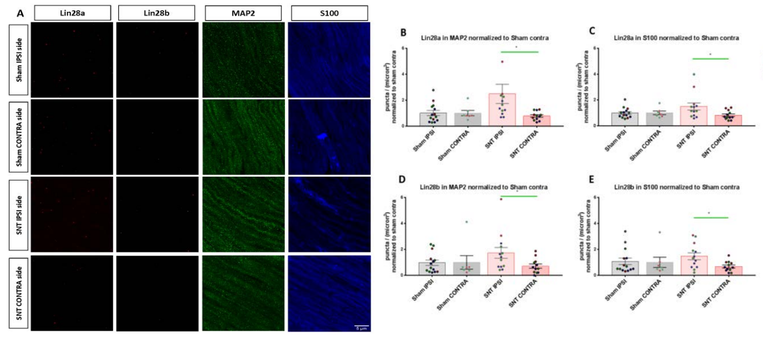
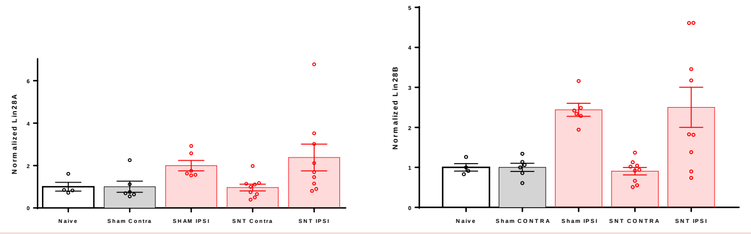


Figure 3: Pilot digital PCR data for Lin28a full gene expression (FGE) in DRG at 3 days post injury (dpi). **(Uche Onuchukwu, Meffert Lab)**. Note: 'sham' is not naïve in this pilot expt.



Future Aims

1. Determine the spatiotemporal timecourse of changes in Lin28 RNAs/proteins and Let-7 miRNAs in dorsal root ganglia (DRG) and sciatic nerve following nerve injury.
2. Investigate the functional role of Lin28 in hyperalgesia following nerve injury.
3. Determine if Lin28 loss of function impacts neuropathic pain through effects on the Let-7 miRNAs.

References

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