A Potential New Target for REST-mediated Chronic Pain

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Abstract

Chronic neuropathic pain originates from the damaged sensory nervous system and remains a major clinical challenge despite multiple clinical studies. Existing literature suggests an altered expression of pain associated genes (PAGs) following nerve injury and involves epigenetic modifications in dorsal root ganglion (DRG). The protein arginine methyltransferase 5 (PRMT5) is predominantly expressed in neuronal cells and catalyzes arginine methylation of histones and many non-histone proteins. However, it remains unclear if such methylation events also occur after nerve injury and pain sensation. Earlier reports suggest an increased expression of the repressor element-1 silencing transcription factor (REST) in DRGs after nerve injury–which contributes to transcriptional repression of various genes and results in the development of chronic pain.

We hypothesize that spared nerve injury (SNI)-induced chronic pain involves REST-mediated silencing of PRMT5. As a first step of this mechanism, we asked whether PRMT5 expression inversely correlates with REST in mice DRG after SNI. We used wild-type and *Rest* conditional knock-out (cKO) mice with various biochemical techniques.

Introduction

Nerve injury leads to many physiological changes including an increase in inflammation and localized sensitivity to various stimuli. Studies have shown that REST is upregulated in sensory neurons after SNI and is necessary for the development of chronic pain. Using a *Rest* cKO mouse line, researchers found reduced levels of chronic pain after SNI when *Rest* was deleted—suggesting that it is a critical factor in the development of chronic pain[2].

REST is a major chromatin modifier which contains a DNA binding domain which binds to the consensus REI sequence. It contains two repressor domains (RD1 and RD2) which bind to many corepressors including Co-REST, HDAC1, 2, etc. These corepressors help REST modify chromatin around its target genes.

Mechanistically, PRMTs are enzymes which catalyze the methylation of various arginine residues within proteins. Catalyzation of arginine residues is made possible through transfers from the S-adenosylmethionine (SAM) substrate which results in monomethylated arginine (MMA) and S-adenosylhomocysteine (SAH). Further, catalyzation of MMA is dependent on the type of PRMT, with Type II enzymes such as PRMT5 being able to catalyze the monomethylation to asymmetric dimethylarginine (sDMA)[3].

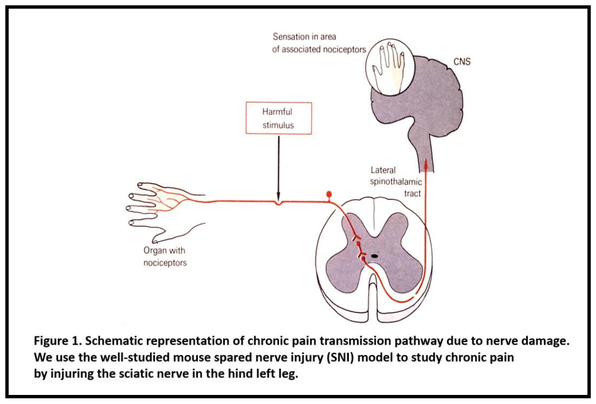

Figure 1. Schematic representation of chronic pain transmission pathway due to nerve damage. We use the well-studied mouse spared nerve injury (SNI) model to study chronic pain by injuring the sciatic nerve in the hind left leg.

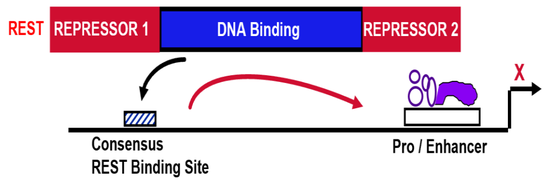

Figure 2: Schematic representation of the transcriptional repressor REST

Methods

The L3-L4 DRGs and spinal cord tissues obtained from wild type (WT) and *Rest* cKO mice before and after SNI on the left limb were used for quantitative real time PCR (qRT-PCR) and western blotting. No SNI (sham) was performed on the right limb of the mice. The REST binding site on the *PRMT5* promoter was bioinformatically predicted by MatInspector (Genomatix).

Results

The initial qPCR results suggested an above 3.5-fold increase of REST mRNA with concurrent reduction in PRMT5 mRNA levels in the SNI compared to uninjured DRGs. The reduction of PRMT5 expression with increased levels of REST in DRGs after SNI was also confirmed by western blotting. In the *Rest* cKO mice, SNI did not affect PRMT5 protein levels, suggesting REST plays a role in regulating PRMT5. The predicted binding site of REST in the mouse *PRMT5* promoter was located at the -369 to -339 region proximal to the transcription start site

Conclusions

Our findings suggest a potential role of REST in negatively regulating PRMT5 expression in the DRGs after SNI. A potential binding site of REST is observed in the *PRMT5* promoter; however, further validation of this binding is needed. Overall, elucidating the role of PRMT5 downregulation in epigenetic alteration of PRGs could unravel a novel mechanism of arginine methylation in chronic pain development.

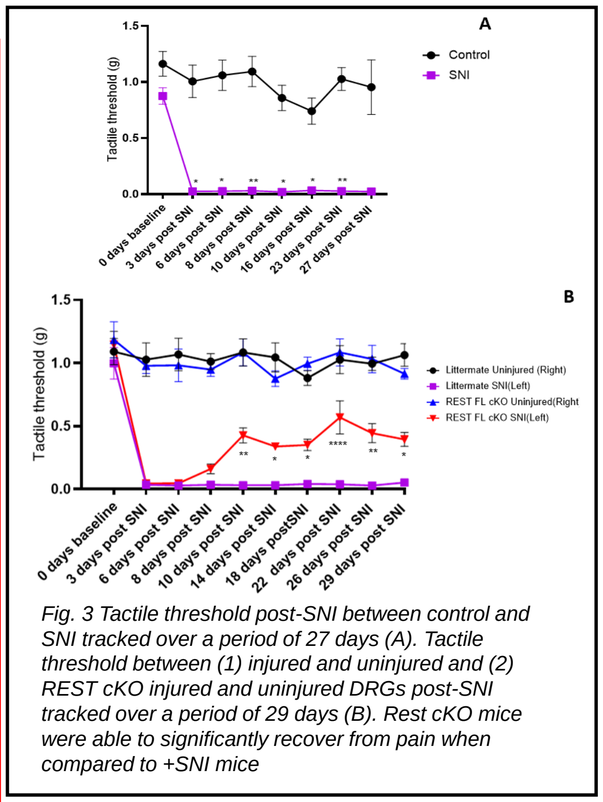

Fig. 3 Tactile threshold post-SNI between control and SNI tracked over a period of 27 days (A). Tactile threshold between (1) injured and uninjured and (2) REST cKO injured and uninjured DRGs post-SNI tracked over a period of 29 days (B). Rest cKO mice were able to significantly recover from pain when compared to +SNI mice

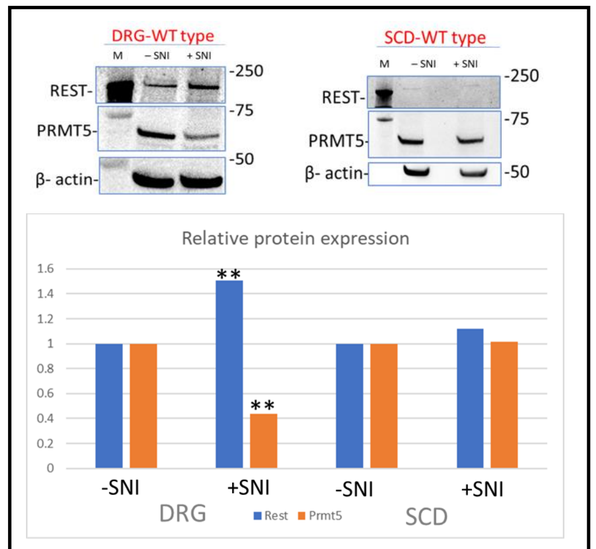

Fig. 4 Quantification of REST and PRMT5 protein levels in the L3 and L4 DRGs and SNI dorsal spinal cord

A significant overexpression of REST observed in injured (+SNI) compared to uninjured (-SNI) ($p<0.01$). Moreover, protein expression of REST and PRMT5 were not altered in the dorsal spinal cord side

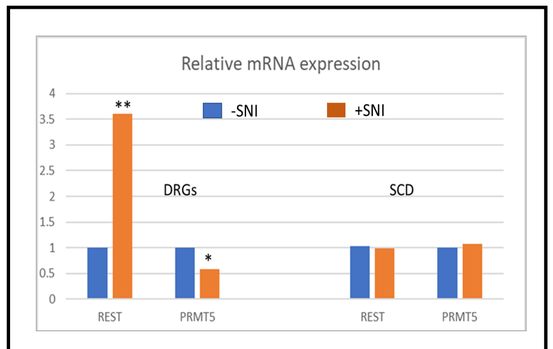

Fig. 5 Relative mRNA expression of REST and PRMT5 between DRGs and SCD

Quantification of mRNA levels of REST and PRMT5 in the L3 and L4 DRGs vs. dorsal side of spinal cord. Significant overexpression of REST is observed in injured (+SNI) compared to uninjured (-SNI) (* $p<0.05$, ** $p<0.01$).

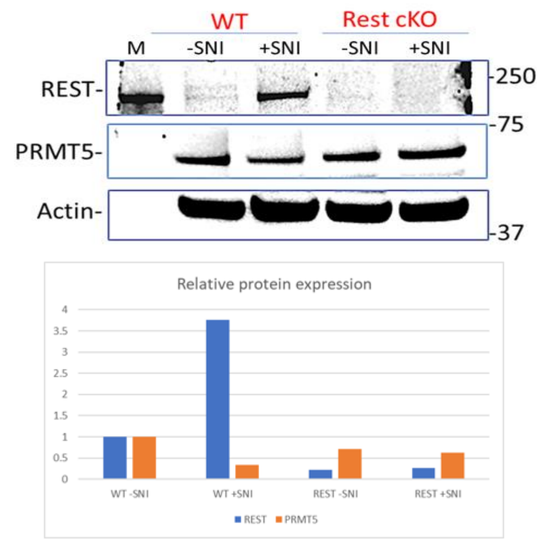

Fig. 6 Quantification of protein expression of REST and PRMT5 levels in the L3 and L4 DRGs from SNI in wild type and REST cKO mice

Here, a significant overexpression of REST was observed in the injured (+SNI) compared to the uninjured (-SNI). Further, down regulation of PRMT5 protein expression between injured DRGs and uninjured DRGs was observed.

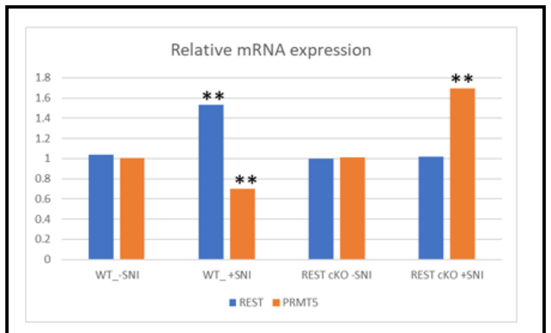

Fig. 7 Quantification of mRNA levels of REST and PRMT5 in the L3 and L4 DRGs

Quantification of mRNA levels shows a significant overexpression of REST and down regulation of PRMT5 being observed in injured (+SNI) compared to uninjured (-SNI) DRGs. Further, +SNI in *Rest* cKO mice caused overexpression of PRMT5 in DRGs.

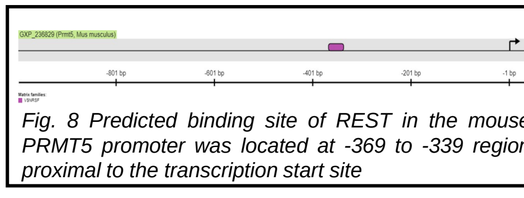

Fig. 8 Predicted binding site of REST in the mouse PRMT5 promoter was located at -369 to -339 region proximal to the transcription start site

References

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