Exosomal cargo containing miR-21 regulates macrophage phenotype via TGF-β

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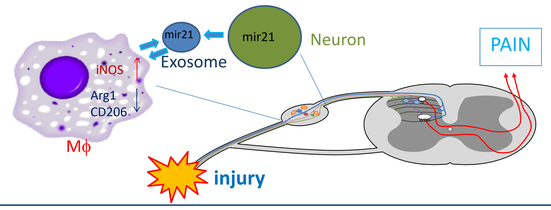
Introduction

At 7 days following peripheral nerve injury the non-coding RNA miR-21 is up-regulated in sensory neuron cell bodies in the dorsal root ganglia (DRG) which release extracellular vesicles containing miR-21 upon activity. Such miR-21 containing exosomes polarise macrophages in the DRG microenvironment towards a pro-inflammatory phenotype and consequently contribute the initiation of nociceptive signalling (Figure 1) (Simeoli et al., Nat Comm. 2017).



Aims

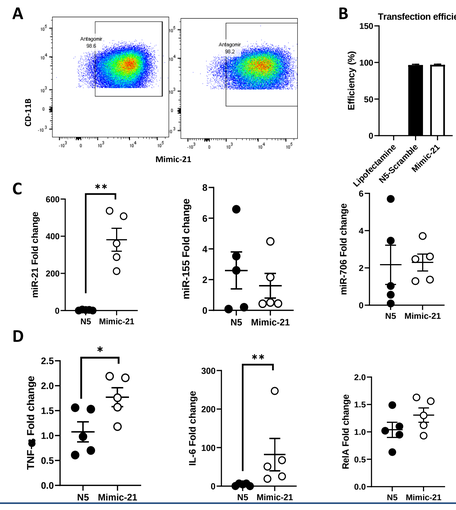
Here we began to investigate which i) miR-21-5p targets modulate macrophage phenotype, and ii) whether neuron-derived miR-21 regulates DRG macrophage phenotype under persistent neuropathic pain

Methods

Peritoneal and bone marrow derived macrophages (BMDM) were transfected with miR-21 mimic or miR-21 antagomir and gene expression measured by qRT-PCR. Spared nerve injury (SNI) was used in WT mice and miR-21 conditional KO in sensory neurons (miR-21 cKO) littermates. Lumbar DRGs were isolated and macrophage phenotype was analysed by qRT-PCR and flow cytometry.

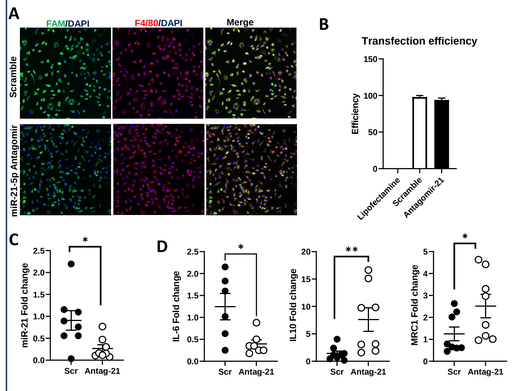
Results

1. miR-21 mimic transfection up-regulates pro-inflammatory cytokines expression in primary macrophages



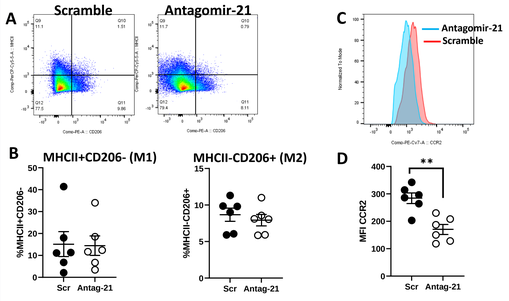
A Scatter plot representing peritoneal macrophages (PM) transfected with miR-21-5p mimic or N5 scramble. **B** Excellent transfection efficiency detected by flow cytometry. **C** Up-regulation of miR-21-5p, but neither miR-155 nor miR-706 expression in miR-21-5p mimic transfected PM **D** Up-regulation of TNF-α, IL-6 and RelA mRNA in miR-21-5p mimic transfected PM. Data are means ± S.E.M, $*P < 0.05$, $**P < 0.01$ Student's *t* test.

2. miR-21 antagomir modulates BMDM phenotype towards M2



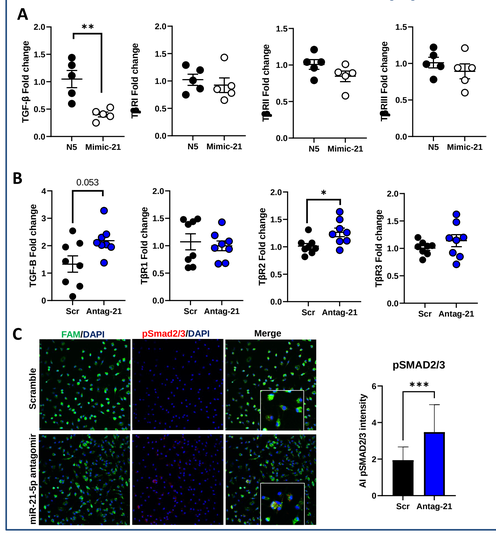
A and **B** Excellent transfection of bone marrow derived macrophages (BMDM) with miR-21-5p antagomir detected by Immuno-cyto-chemistry **C** Down-regulation of miR-21-5p expression in BMDM at 48h after transfection with miR-21 antagomir (Antag-21) compared to scramble (Scr). **D** down-regulation of IL-6 and up-regulation of IL-10 and Mrc1 mRNA levels in miR-21 antagomir transfected BMDM
Data are mean ± SEM, n=8 (** P<0.01) Student's *t* test

3. miR-21 antagomir regulates CCR2 receptor expression



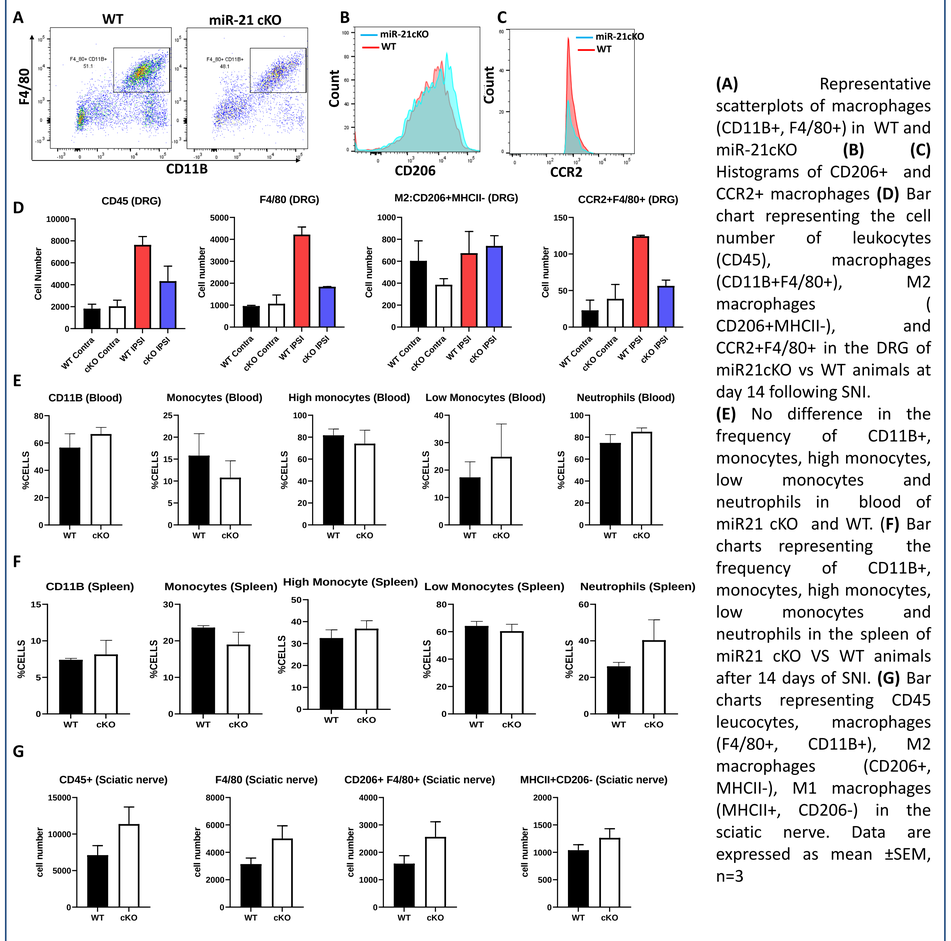
A Representative scatterplots of BMDM transfected with scramble or miR-21 antagomir immunolabeled with MHCII and CD206. **B** No changes in M1 (MHCII+CD206-) and M2 (MHCII-CD206+). **C** and **D** down-regulation of CCR2 expression in BMDM after transfection with the antagomir. Data are mean ± SEM, n=6 (** P<0.01).

4. miR-21 modulates the TGF-β pathway in macrophages



A In PM, miR-21 mimic transfection down-regulates mRNA levels for TGF-β, but not TβRI, TβRII, TβRIII **B** in BMDM, miR-21 antagomir transfection up-regulates TGFβ, and TβRII but not TβR1, and TβRII. **C** and **D** In BMDM, miR-21 antagomir transfection up-regulates pSmad2/3 expression. Data expressed as mean ±SEM. (**P<0.01). Scale bar equals to 50 µm.

5. miR-21 conditional deletion in sensory neurons is associated with reduced macrophage infiltration in DRG at 14 days after nerve injury



(A) Representative scatterplots of macrophages (CD11B+, F4/80+) in WT and miR-21cKO **(B) (C)** Histograms of CD206+ and CCR2+ macrophages **(D)** Bar chart representing the cell number of leukocytes (CD45), macrophages (CD11B+F4/80+), M2 macrophages (CD206+MHCII-), and CCR2+F4/80+ in the DRG of miR21cKO vs WT animals at day 14 following SNI.
(E) No difference in the frequency of CD11B+, monocytes, high monocytes, low monocytes and neutrophils in blood of miR21 cKO and WT. **(F)** Bar charts representing the frequency of CD11B+, monocytes, high monocytes, low monocytes and neutrophils in the spleen of miR21 cKO VS WT animals after 14 days of SNI. **(G)** Bar charts representing CD45 leucocytes, macrophages (F4/80+, CD11B+), M2 macrophages (CD206+, MHCII-), M1 macrophages (MHCII+, CD206-) in the sciatic nerve. Data are expressed as mean ±SEM, n=3

Conclusions

MiR-21-5p silencing in DRG neurons is associated with a lower number of CCR2+ macrophages compared to control neuropathic DRG. MiR-21-5p silencing in macrophages promotes an M2-phenotype through activation of the TGF-β pathway. We suggest that the delivery of miR-21 antagomir would reverse persistent neuropathic allodynia by polarising macrophages towards an antinociceptive phenotype.