Neonatal sEVs delivering miR-487b-3p break a metabolic-immune vicious cycle to promote scarless spinal cord repair

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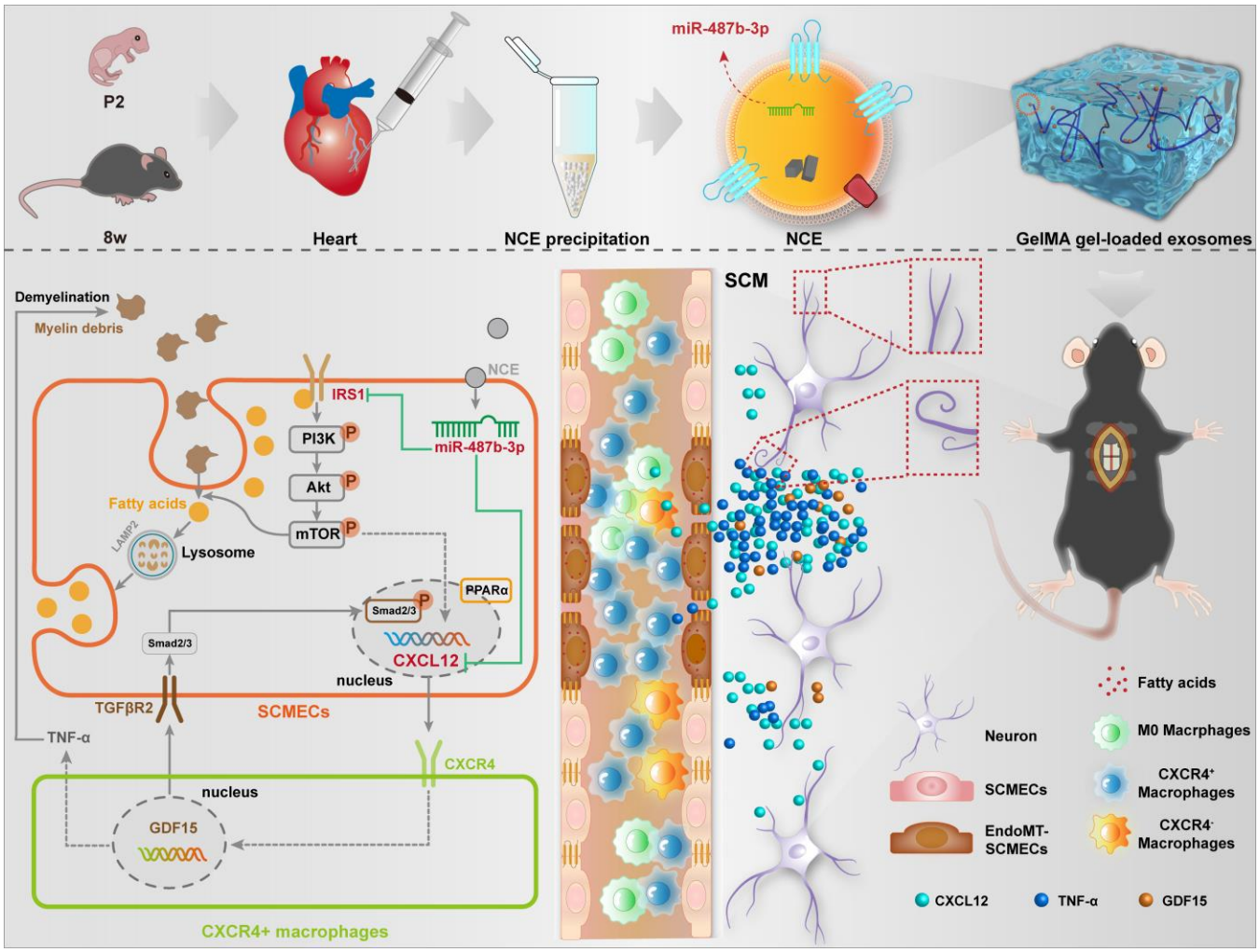
INTRODUCTION: Spinal cord injury (SCI) in adult mammals results in permanent disability due to failed regeneration and scar formation, whereas neonatal mice achieve scarless repair. Here, we report that small extracellular vesicles (sEVs) from neonatal mouse blood (NCE) rejuvenate adult spinal cord microvascular endothelial cells (SCMECs), rescuing lipid metabolic homeostasis, suppressing immune dysregulation, and enabling neuroaxonal regrowth. Mechanistically, myelin debris post-SCI triggers IRS1-mediated PI3K-Akt-mTOR hyperactivation in SCMECs, inducing pathogenic lipid droplet accumulation, lysosomal/mitochondrial dysfunction, and mesenchymal transition. Multi-omics analysis revealed that mesenchymal SCMECs secrete CXCL12 to recruit CXCR4+ macrophages, which release TNF-α and GDF15, exacerbating immune dysfunction and fibrotic scarring. Neonatal sEVs deliver high levels of miR-487b-3p, which silences both IRS1 and CXCL12, breaking this vicious cycle. This restores microvascular integrity, reduces scarring, and promotes functional recovery after SCI. Our findings unveil a metabolic-immunological axis impairing adult SCI repair and identify miR-487b-3p-enriched sEVs as a promising therapeutic strategy.

METHODS: sEVs were isolated from postnatal day 2 (NCE) and adult (ACE) mouse plasma and characterized by NTA, TEM, and Western blot (WB). For in vivo therapy, sEVs were embedded in GelMA hydrogel and applied to the lesion site of a mouse SCI model. Functional recovery was assessed using the Basso Mouse Scale (BMS), Louisville Swim Scale (LSS), and motor-evoked potentials (MEP). Histological evaluations (scar composition, axonal regeneration via BDA tracing) and immunohistochemistry were performed. In vitro, spinal cord microvascular endothelial cells (SCMECs) were treated with myelin debris with or without sEV pre-treatment to assess lipid metabolism (BODIPY/PLIN2 staining), EndoMT (α-SMA/Collagen I), and inflammatory activation (VCAM-1). miRNA sequencing identified miR-487b-3p as a key candidate, and its role was validated via gain-/loss-of-function experiments. Targets were confirmed by dual-luciferase assay and rescue experiments.

RESULTS SECTION: **1. NCE Promotes Functional and Structural Recovery**: NCE treatment significantly improved locomotor function (BMS score: 5.2 ± 0.3 vs. 2.8 ± 0.4 in ACE group; $p < 0.01$), reduced lesion size, and enhanced axonal regeneration across the lesion core. Histology showed decreased deposition of fibrotic scar components (Collagen I, Fibronectin, Laminin). **2. NCE Rescues Lipid Metabolism and Suppresses EndoMT**: In vitro, NCE attenuated myelin debris-induced lipid droplet accumulation in SCMECs and inhibited EndoMT transition (reduced α-SMA and Collagen I expression). **3. miR-487b-3p Mediates Therapeutic Effects**: miRNA-seq revealed miR-487b-3p as highly enriched in NCE. It directly targeted **Irs1**(inhibiting PI3K-Akt-mTOR-driven lipid synthesis) and **Cxcl12**(a chemokine for macrophage recruitment). Inhibiting miR-487b-3p abolished NCE's benefits, while its overexpression mimicked NCE effects. **4. Breaking the Metabolic-Immune Vicious Cycle**: Myelin debris-activated SCMECs secreted CXCL12 to recruit CXCR4+ macrophages, which secreted GDF15 to further exacerbate EndoMT via TGFβR2. NCE-derived miR-487b-3p simultaneously suppressed IRS1 and CXCL12, disrupting this cycle. **5. Therapeutic Translation**: Synthetic miR-487b-3p mimic (Ago-miR) delivered via GelMA hydrogel recapitulated the benefits of NCE in vivo.

DISCUSSION: This study identifies neonatal sEVs as key mediators of scarless regeneration and unveils a pathological metabolic-immune vicious cycle in adult SCI involving SCMECs and macrophages. The dual targeting of IRS1 and CXCL12 by miR-487b-3p provides a novel strategy to simultaneously regulate lipid metabolism and immune response. Limitations include the focus on a single miRNA and the use of rodent models.

SIGNIFICANCE/CLINICAL RELEVANCE: This work provides a novel cell-free therapeutic strategy using defined neonatal sEVs or synthetic miRNA mimics to achieve scarless repair. By elucidating the miR-487b-3p/Irs1/Cxcl12 axis, it identifies precise targets to disrupt pathological cross-talk between endothelial and immune cells, offering potential for treating SCI and other fibrosis-related disorders.


P2
8w
Heart
NCE precipitation
miR-487b-3p
NCE
GelMA gel-loaded exosomes
Demyelination
Myelin debris
NCE
IRS1
PI3K
Akt
mTOR
miR-487b-3p
Fatty acids
LAMP2
Lysosome
PPARα
Smad2/3
Smad2/3
CXCL12
nucleus
TGFβR2
SCMECs
TNF-α
CXCR4
nucleus
GDF15
CXCR4+ macrophages
SCM
Fatty acids
Neuron
M0 Macrphages
SCMECs
CXCR4+ Macrophages
EndoMT-SCMECs
CXCR4- Macrophages
CXCL12
TNF-α
GDF15