Vagus Nerve Schwann Cell Erythropoietin Receptors are Critical for Early Functional Recovery of Intestinal Motility after Postoperative Ileus

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Introduction

- Intestinal motility disorders (IMDs) are poorly understood and medically complex problems affecting an increasing number of patients.
- Postoperative ileus (POI) is a form of IMDs with frequent occurrence after abdominal surgery.
- Erythropoietin (EPO) is a multifunctional tissue-protective cytokine that promotes recovery of the intestine in various injuries.
- While EPO receptors (EPOR) are present on vagal Schwann cells, the role of EPOR in POI recovery is unknown because of the lack of EPOR antagonists or Schwann-cell specific EPOR knockout animals.
- This study was designed to explore the effect of EPO via EPOR on vagus nerve Schwann cells in a mouse model of POI.

Methods

- We used the Cre-loxP system to develop a myelin protein zero (Mpz) promoter-driven knockout mouse model of vagus nerve-Schwann cell EPOR (MpzCre-EPOR$^{flox/flox}$ / Mpz-EPOR-KO) and confirmed using genotyping PCR and qRT-PCR.
- Mpz-EPOR-KO and control mice were assigned to POI followed by EPO treatment.
- We then measured the intestinal transit time at baseline and after induction of POI with and without EPO treatment.

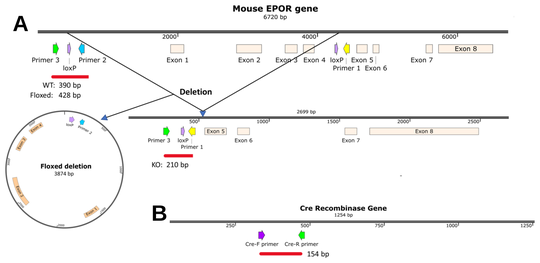

Figure 1. Generation of Schwann cell-specific Mpz-EPOR-KO mice. A, B. Scheme showing Mpz pomoter-driven Cre recombinase selectively cut EPOR gene at the loxP sites within vagus nerve Schwann cells resulting in a 4021-bp deletion spanning exons 1 through 4 of the EPOR gene.

Results

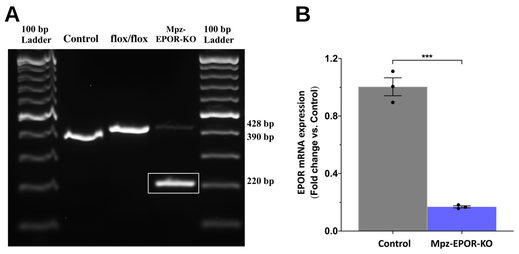

Figure 2. Verification of vagus nerve specific Mpz-EPOR-KO in mice. A. PCR genotyping with DNA isolated from the vagus nerve revealed the presence of the 220-bp PCR product, resulting from MpzCre mediated recombination in the Schwann cell of MpzCre-EPOR$^{flox/flox}$ mice and not in wild-type (control; 390 bp) and flox/flox (428 bp) mice (n =3). **B.** qRT-PCR analysis confirms a significant knockout of the EPOR gene in the vagus nerve as compared to control mice. means ± SEM, *** P < 0.0002, n =3.

Results

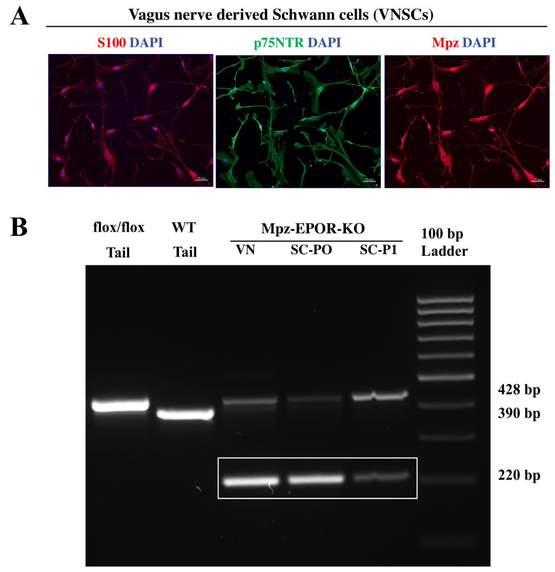

Figure 3. Verification of vagus nerve Schwann cell-specific Mpz-EPOR-KO in mice. A. Characterization of mouse vagus nerve derived Schwann cells (VNSCs). The identity and purity of SCs were confirmed using IF staining of S100, p75NTR, and Mpz under a fluorescent microscope (ZEISS Apotome 2). The purity of the cultured SCs (99%) was analyzed by double positive staining of DAPI with S100/p75NTR/Mpz markers from 3 independent experiments. Each image represents 3 images from 3 independent experiments. Scale bar: 50 µm, n =3. **B.** PCR genotyping with DNA isolated from the vagus nerve and VNSCs (passage zero, SC-P0; passage one, SC-P1) revealed the presence of the 220-bp PCR product, resulting from MpzCre mediated recombination in the Schwann cell of MpzCre-EPOR$^{flox/flox}$ mice and not in wild-type (control; 390 bp) and flox/flox (428 bp) mice (n =3).

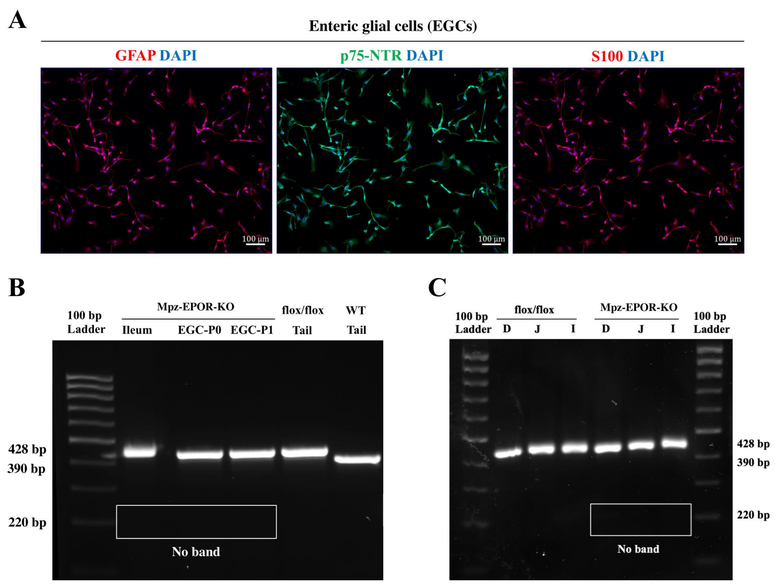

Figure 4. Verification of enteric glial cell-specific Mpz-EPOR-KO in mice. A. Characterization of mouse enteric glial cells (EGCs). The identity and purity of SCs were confirmed using IF staining of GFAP, p75NTR, and S100 under a fluorescent microscope (ZEISS Apotome 2). The purity of the cultured EGCs (99%) was analyzed by double positive staining of DAPI with GFAP/p75NTR/S100 markers from 3 independent experiments. Each image represents 3 images from 3 independent experiments. Scale bar: 100 µm, n =3. **B.** PCR genotyping with DNA isolated from EGCs (passage zero, EGC-P0; passage one, EGC-P1) revealed no deletion of EPOR in MpzCre-EPOR$^{flox/flox}$ mice. EPOR bands of wild-type (control; 390 bp) and flox/flox (428 bp) tail samples of mice are depicted (n =3). **C.** PCR genotyping with DNA isolated from the segments of the intestine (D, duodenum; J, jejunum; I, ileum) revealed no deletion of EPOR in MpzCre-EPOR$^{flox/flox}$ mice (n =3).

Results

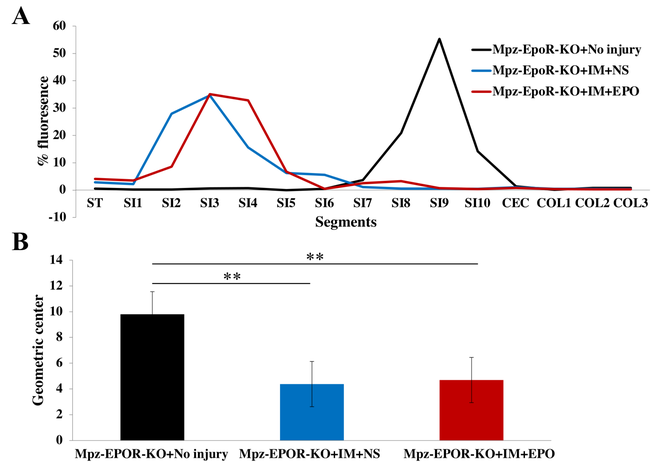

Figure 5. Role of EPO on intestinal transit time following POI. A. The percent fluorescence of the stomach (ST), ten segments of the small intestine (SI 1–10), cecum (CEC), and three segments of the colon (COL 1–3). **B.** Gastrointestinal transit geometric center. Black graph (control, no manipulation), blue graph (IM + saline), and red graph (IM + EPO) treatment group. One-way ANOVA, Tukey's multiple comparisons test. Data were expressed as means ± SEM, **P< 0.0021, No injury vs. saline vs. sham; n = 4 /group. NS, Normal Saline.

Conclusions

- This is the first pre-clinical study to demonstrate a novel mouse model of EPOR specific knock out on Schwan cells with an effect in the gut.
- We also showed novel beneficial effects of EPO through vagus nerve Schwann cell-EPOR in intestinal dysmotility.
- Our findings suggested that EPO-EPOR signaling in the vagus nerve after POI is critical for the functional recovery of intestinal transit time.

Future Directions

- Our research will involve studying the effects of EPO-EPOR neuronal and cellular signaling on intestinal transit following POI using our established mouse model of vagus nerve Schwann cells EPOR knockout.

Acknowledgements

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Reference

- Govindappa PK, Begom M, Gupta Y, Elfar JC, Rawat M, Elfar W. A critical role for erythropoietin on vagus nerve Schwann cells in intestinal motility. BMC Biotechnol. 2023 May 1;23(1):12. doi: 10.1186/s12896-023-00781-x. PMID: 37127673; PMCID: PMC10152589.

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