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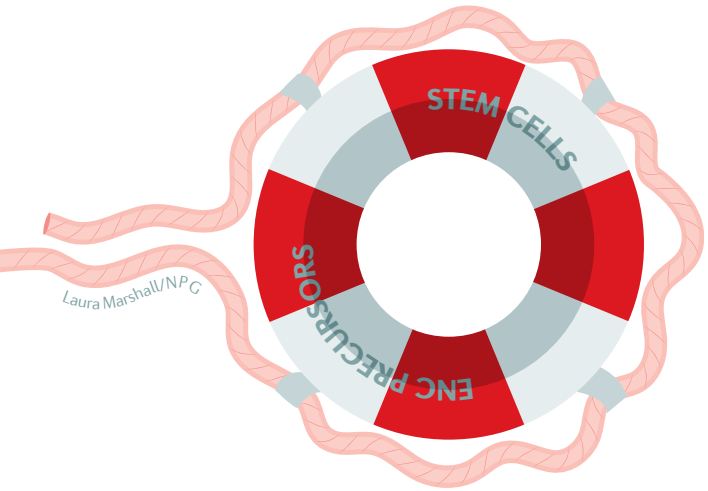
ENTERIC NERVOUS SYSTEM

Modelling and treating Hirschsprung disease: stem cells to the rescue?

> Alternative therapeutic approaches for Hirschsprung disease are urgently needed…

Researchers have derived enteric neural crest (ENC) precursors from human stem cells that can be further differentiated into functional enteric neurons that rescue disease-related mortality in a mouse model of Hirschsprung disease. The study, reported in *Nature*, details a potential new approach to model and treat Hirschsprung disease.

Hirschsprung disease is an example of a developmental disorder of the enteric nervous system (ENS), in which neural-crest-derived cells fail to colonize the distal intestine, resulting in missing enteric neurons (aganglionosis) and lack of function. The standard treatment is surgical removal of the defective section, but complications can arise and, for those with severe disease, so little of the bowel can remain that intravenous nutrition is required for survival. Alternative therapeutic approaches for Hirschsprung disease are urgently needed and regenerative medicine might offer a solution. "We were interested in a system where we can show the power of stem-cell based approaches for both regenerative medicine (via transplantation) and drug discovery (*in vitro* drug screening)," explains author Lorenz Studer.



Human pluripotent stem cells (hPSCs) formed the basis of the researchers' experimental approach. Their experiments had three core steps: development of a stem-cell differentation protocol, regenerative medicine and disease modelling. "Most past studies on the ENS were based on the use of primary ENC precursors isolated from the developing embryo," explains Studer. "Here we can generate human ENC from scratch using hPSCs."

The investigators developed a protocol to generate ENC precursors from hPSCs, showing that addition of retinoic acid could switch cells to an ENC identity. The ENC precursors could then be further differentiated into functional enteric neurons. When transplanted into a developing chick embryo, these *in-vitro*-derived ENC precursors migrated along the trunk of the embryo and colonized the gut. Interestingly, the ENC precursors differentiated into enteric neurons with remarkable diversity in terms of subtypes, with a broad range of neurotransmitter phenotypes, including serotonin-positive, γ-aminobutyric-acid-positive and nitric-oxide-synthase-positive neurons.

Crucially, *in vivo* engraftment and migration of hPSC-derived ENS precursors was demonstrated. When transplanted into mice, human ENC precursors migrated extensively and had repopulated the entire length of the colon after 2–4 weeks. Importantly, in a mouse model of Hirschsprung disease (bearing mutations in *Ednrb*, a known genetic cause of Hirschsprung disease), transplantation of human ENS precursors prevented death from the disease; most control mice died (with megacolon-like pathology) but all mice injected with ENS precursors survived. Preliminary findings showed a trend towards improved gastrointestinal transit time in treated mice.

Finally, the *in-vitro*-derived ENS precursors were used as a model for Hirschsprung disease and a platform for drug screening. CRISPR-Cas-based gene-editing techniques were used to create *EDNRB*-null ENC precursors, which were then used to perform a small-molecule screen (consisting of 1,280 FDA-approved drugs) to identify compounds capable of restoring ENC migration. Using this approach, pepstatin A was identified as a candidate therapeutic target.

"The migratory behaviour of the [ENC precursor] cells … suggests that it may be possible to attempt the restoration of severe ENS conditions such as total aganglionosis," notes Studer, although he cautions that further work is needed to better understand the extent of integration of the cells and their long-term behaviour, especially in a more physiological setting.

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ORIGINAL ARTICLE Fattahi, F. *et al.* Deriving human ENS lineages for cell therapy and drug discovery in Hirschsprung disease. *Nature* http://dx.doi.org/10.1038/nature16951
FURTHER READING Obermayr, F. *et al.* Development and developmental disorders of the enteric nervous system. *Nat. Rev. Gastroenterol. Hepatol.* **10**, 43–57 (2012)