News Release

Title

Dental pulp stem cells as a therapy for congenital entero-neuropathy:

~~ Regeneration of gut motility using multipotent stem cells

Key Points

○ Hirschsprung's disease (HSCR) and its allied disorders are congenital entero-neuropathies that occur with an incidence of ~1 per 5000 livebirths in Japan. The current management of HSCR involves surgery to remove or bypass affected segments of the bowel, but many children suffer from life-long complications. We thus explored a novel cell-based therapy for entero-neuropathies.

○ Dental pulp stem cells derived from deciduous teeth (dDPSCs) are multipotent cells that express NCC markers and have low levels of class II HLA. Japanese Fancy-1 (JF1) mouse is an animal model of entero-neuropathy due to *Ednrb* mutation, showing a sparse network of myenteric ganglia, especially in the proximal colon. Intravenous transplantation of human dDPSCs improved the survival and nutritional status of JF1 mice.

○ The proximal colon of wild-type B6 mice displayed basal electric oscillations with a period of 3-4 s, whereas electrical complexes of rapid and slow potentials occurred in JF1 mouse colon. Transplantation of human dDPSCs into JF1 mice restored basal electric oscillations similar to wild-type mice.

○ In JF1 mice, transplanted human dDPSCs migrated to affected regions of the intestine, and differentiated into both pacemaker cells and enteric neurons in the proximal colon. In addition, the protein levels of mouse NGF and GDNF and human GDNF, NGF and SCF increased, being consistent with paracrine actions of dDPSCs.

○ As Hirschsprung (1888) and Bayliss and Starling (1899) consistently presented the clinical and physiological evidence, respectively, the notion that intrinsic nervous system coordinates intestinal motility has become established. Our cell-therapy provides the evidence that complex intestinal movements are actually organized by the cooperation of multiple motor systems, including the network of pacemaker cells.

Summary 1

Assoc. Prof. Shinsuke Nakayama and Research Associates (Ms Naoko Iwata, and Chiho Takai)

at the Department of Cell Physiology, Nagoya University Graduate School of Medicine (Dean: Kenji Kadomatsu) demonstrated that dental pulp stem cells derived from deciduous teeth (dDPSCs) have potential for development into a new treatment for congenital entero-neuropathies, using an animal model. This was achieved through collaborative research work with Kyushu University, Fukuoka Dental College, and International University of Health and Welfare. The results of this stem cell therapy also validated that multiple motor systems are operated to coordinate complex movement of the gut.

Hirschsprung's disease (HSCR) and its allied disorders are congenital entero-neuropathies that occur with an incidence of ~1 per 5000 livebirths in Japan. The current management of HSCR involves surgery to remove or bypass affected segments of the bowel. Nevertheless, many children suffer from life-long complications due to the dysfunction of 'healthy' bowel retained during surgery, etc.

We thus explored a novel cell-based therapy. dDPSCs are multipotent cells that express NCC markers and have low levels of class II HLA. Japanese Fancy-1 (JF1) mouse is an animal model of entero-neuropathy due to *Ednrb* mutation, showing a sparse network of myenteric ganglia, especially in the proximal colon. Intravenous transplantation of human dDPSCs improved the survival and nutritional status of JF1 mice (Fig. 1).

The proximal colon of wild-type B6 mice displayed basal electric oscillations with a period of 3-4 s, whereas electrical complexes of rapid and slow potentials frequently occurred in JF1 mouse colon. Superbly, transplantation of human dDPSCs into JF1 mice restored basal electric oscillations similar to wild-type mice (Fig. 2). In JF1 mice, transplanted human dDPSCs migrated to affected regions of the intestine through the interactions between SDF1α and CXCR4, and differentiated into both pacemaker cells and enteric neurons (Fig. 1c). In addition, the protein levels of human GDNF, NGF, SCF etc. significantly increased, indicating their paracrine actions.

The clinical and physiological evidence presented by Hirschsprung (1888) and Bayliss and Starling (1899), respectively, logically match strongly. Thereafter, it has become established that intestinal motility is coordinated by an intrinsic nervous system. Seemingly, this notion has too much affected medical treatment guidelines, even after the discovery of interstitial cells of Cajal (ICCs) that act as an intestinal pacemaker by the end of the 20th century. Our transplantation study using multipotential stem cells (dDPSCs) has presented the evidence that complex intestinal movements actually require the cooperation of multiple motor systems, including network-forming pacemaker cells of ICCs. We anticipate that our study leads the current medical guidelines for intestinal motility disorders to be modified appropriately, and promote the development of radical therapy.

This study was published in an online multidisciplinary, open access journal "Scientific Reports".

Summary 2

Novel therapies are urgently needed for congenital entero-neuropathies, including

Hirschsprung's disease (HSCR). We thus examined the therapeutic potential of dental pulp stem cells derived from deciduous teeth (dDPSCs). Intravenous transplantation of human dDPSCs improved the survival and nutritional status of Japanese Fancy-1 (JF1) mouse, which is an animal model of entero-neuropathy due to *Ednrb* mutation. This treatment restored basal electric oscillations in the proximal colon of JF1 mice. Transplanted human dDPSCs migrated to affected regions of the intestine, and differentiated into both pacemaker cells and enteric neurons.

Research Background

HSCR and its allied disorders are congenital entero-neuropathies that occur with an incidence of ~1 per 5000 livebirths in Japan. The current management of HSCR involves surgery to remove or bypass affected segments of the bowel. Nevertheless, many children suffer from life-long complications due to the dysfunction of 'healthy' bowel retained during surgery, etc. Therefore, novel therapies are urgently needed.

GI motility research has a long history. Hirschsprung, Danish physician, described a congenital megacolon in the case of two infants with local defects of enteric neurons in 1888. The pathogenesis of HSCR is still incompletely understood but thought to involve the failure of enteric neural crest-derived cells (NCCs) to complete their colonization of the distal intestine during foetal development. On the other hand, from the discovery in the animal experiment that enteric neural circuits play an essential role in peristaltic movements of the small intestine induced by local stimuli, Bayliss and Starling, UK physiologists, proposed the 'law of the intestine' (1899). Since then, it has become established that intestinal motility is coordinated by an intrinsic nervous system. Has this notion too much affected current medical treatment guidelines? In the 1990s, the interstitial cells of Cajal (ICCs) were identified as specialized cells that act as an intestinal pacemaker. In addition, ICCs (especially those in the myenteric plexus) are thought to contribute to the spatial organization of gut motility because of their network-like structure.

Research Results

In this study, we explored a novel cell-based therapy, using three types of stem cells, including dDPSCs, which are multipotent cells that express NCC markers and have low levels of class II HLA. Japanese Fancy-1 (JF1) mouse is an animal model of entero-neuropathy due to *Ednrb* mutation, showing a sparse network of myenteric ganglia, especially in the proximal colon.

Intravenous transplantation of human dDPSCs improved the survival and nutritional status of JF1 mice, accompanied by improved intestinal motility (Fig. 1). The proximal colon of wild-type B6 mice displayed basal electric oscillations with a period of 3-4 s, whereas electrical complexes of rapid and slow potentials frequently occurred in JF1 mouse colon (Fig. 2). The results suggest that loss of spontaneous rhythmicity generated by network-forming pacemaker cells is compensated for by the relatively preserved enteric neurons in the proximal colon of JF1 mice.

Transplantation of human dDPSCs into JF1 mice restored basal electric oscillations similar to wild-type mice (Fig. 2). This treatment also restored spontaneous contraction of JF1 mouse proximal colon. In JF1 mice, transplanted human dDPSCs migrated to affected regions of the intestine through interactions between stromal cell-derived factor-1α (SDF1α) and C-X-C chemokine receptor type-4 (CXCR4), and differentiated into both pacemaker cells and enteric neurons (Fig. 1c). In addition, the protein levels of mouse NGF and GDNF and human GDNF, NGF and SCF increased, being consistent with paracrine actions of dDPSCs.

Our research findings were published in an online multidisciplinary, open access journal "Scientific Reports".

Research Summary and Future Perspective

Our findings imply that congenital entero-neuropathies involve defects of the intestinal pacemaker system and that dDPSCs have potential for development into a new treatment for HSCR in humans. The pacemaker and intrinsic nervous systems of the proximal colon are impaired in JF1 mice with *Ednrb* mutation. Therefore, dDPSCs appropriately worked in this model of gut motility disorder due to their multi-lineage potential as well as their paracrine effects. Further investigations are required to optimize the multilineage potential of stem cells and the transplantation technique, including model animal experiments for distinct types of motility disorders.

We anticipate that this study will be an opportunity to modify the explanation for mechanisms underlying coordinated movement of the gut in medical textbooks and also to develop a radical treatment for intractable intestinal motility disorders including entro-neuropathies.

Publication

Koichiro Yoshimaru, Takayoshi Yamaza, Shunichi Kajioka, Soichiro Sonoda, Yusuke Yanagi, Toshiharu Matsuura, Junko Yoshizumi, Yoshinao Oda, Naoko Iwata, Chiho Takai, Shinsuke Nakayama*, Tomoaki Taguchi (2022). Dental pulp stem cells as a therapy for congenital entero-neuropathy. *Scientific Reports* **12**, 6990.
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Japanese ver.
https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/Sci_220511.pdf

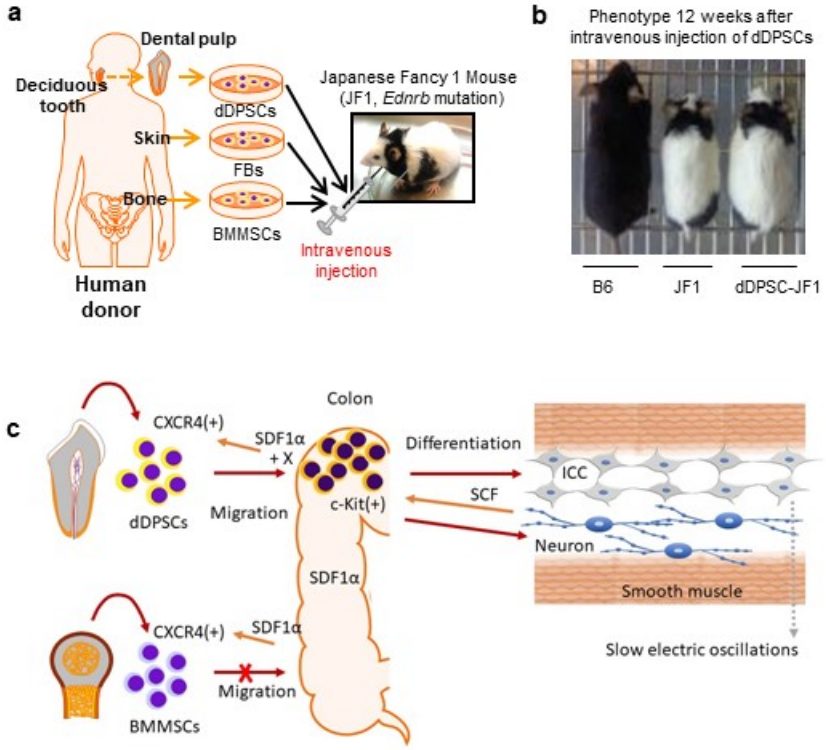

Figure 1: Stem cell therapy. **a**, Transplantation of human deciduous dental pulse stem cells (dDPSCs), bone marrow mesenchymal stem cells (BMMSCs) and skin fibroblasts (FBs). **b**, Comparison of wild-type B6, JF1 and dDPSC-JF1 mice (12 weeks after dDPSC transplantation for dDPSC-JF1 mice). **c**, Schematic diagram showing the possible mechanisms underlying dDPSC migration, differentiation and beneficial effects on colonic motility in JF1 mice.

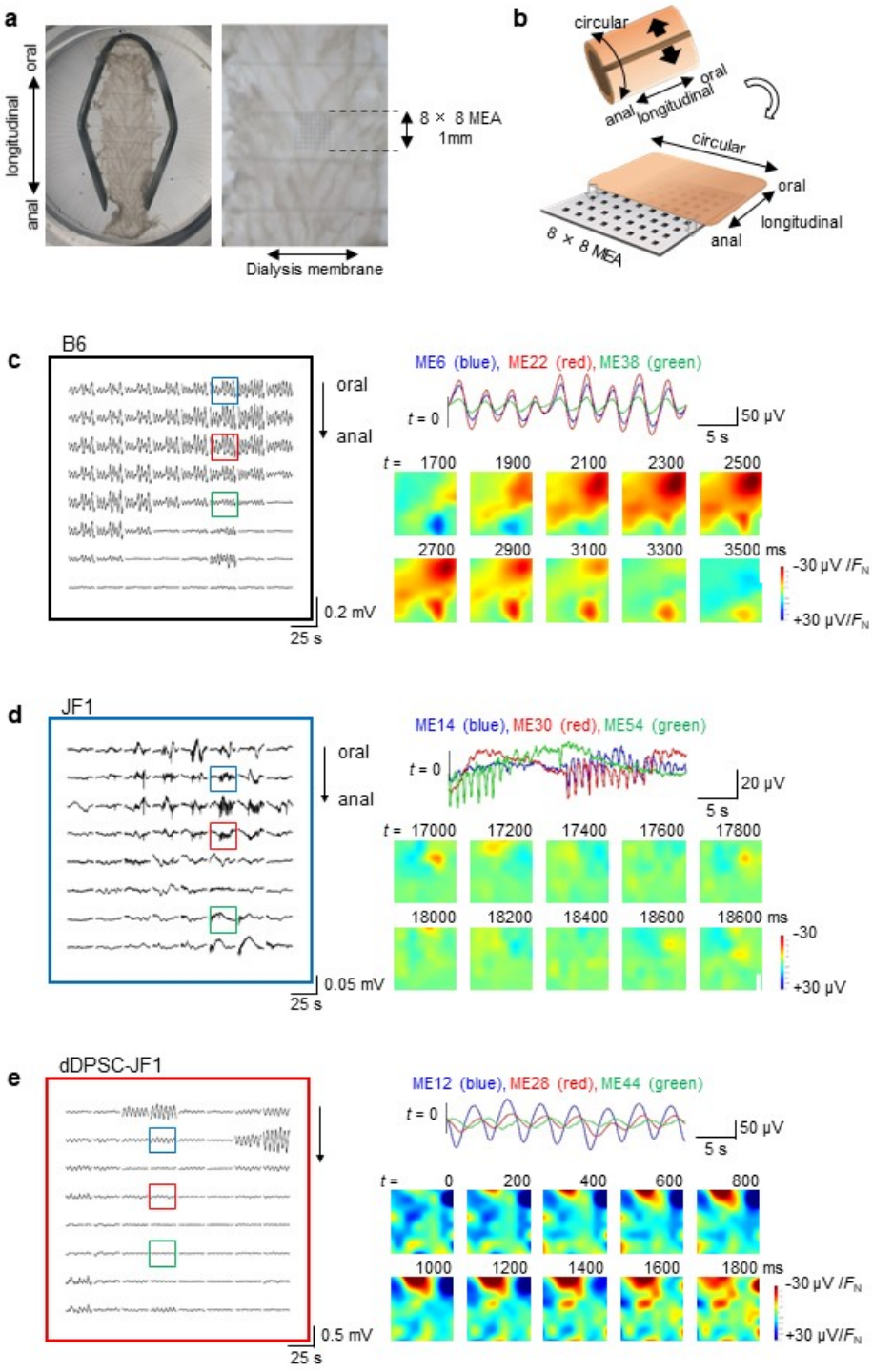

Figure 2: Electric field potentials.

a, Recording of electrical potentials from a sample of proximal colon muscle using an 8×8 MEA. **b**, Procedures used for sample preparation. The proximal region of the colon was cut along the mesentery, and the mucosa was removed. The enteric muscle sample was mounted (longitudinal muscle layer facing downward) on an 8×8 MEA (interpolar distance = 150 µm). **c–e**, Field potentials recorded from the proximal colon of wild-type (B6), JF1 and dDPCS-transplanted JF1 mice using an 8×8 MEA and the corresponding reconstructed potential maps