Press Release

Title:

Transplantation of Neural Crest-Like Cells Derived from Induced Pluripotent Stem Cells Improves Diabetic Polyneuropathy in Mice.

Highlights:

- Impaired vascularity and nerve degeneration are the most important pathophysiological abnormalities of diabetic polyneuropathy (DPN).
- The neural crest (NC) is a transient embryonic structure in vertebrates that differentiates into a vast range of cells, including peripheral neurons, Schwann cells and vascular smooth muscle cells.
- The research group investigated the ability of transplantation of NC like (NCL) cells derived from aged mouse induced pluripotent stem (iPS) cells in the treatment of DPN.
- iPS cells were induced to differentiate into neural cells by stromal cell-derived inducing activity (SDIA) and subsequently supplemented with bone morphogenetic protein 4 to promote a differentiation of NC lineage.
- Impaired nerve functions in diabetic mice were significantly ameliorated by the transplantation of NLC cells.

Summary:

Tetsuji Okawa (Doctor course student, 4th year) and Ken-ichi Isobe, MD, PhD (Professor) et al., at Nagoya University Graduate School of Medicine (Dean:Masahide Takahashi, MD, PhD) discovered that transplantation of Neural Crest Cells derived from aged mice iPSCs improved Diabetic Polyneuropathy. The research results have been published in Cell Transplantation : The Regenerative Medicine Journal. Editors: Camillo Ricordi & Shin-Zong Lin 2012 Oct 8. [Epub ahead of print]

1. Research Background

Diabetic polyneuropathy (DPN) is the most common and intractable complication of diabetes. Loss of sensation in the lower limbs at the advanced stage of DPN is a high risk factor for limb amputation. The major pathophysiological characteristics of DPN are degeneration of nerve fibers and reduced nerve blood flow. Therefore, cells that can express both angiogenic and neurotrophic factors would be suitable for cell transplantation therapy in DPN, and it would be more appropriate if the transplanted cells could differentiate into vascular cells or neural cells. The generation of induced pluripotent stem cells (iPSCs) open the possibility to personalized cell therapy. When applying the iPSCs for DNP therapy, it is necessary to establish the iPSCs from elderly patients themselves. Recently we established iPSCs from aged C57BL/6 mice carrying pCAG-EGFP.

We hypothesized that NCL cells derived from iPS cells would be suitable for cell transplantation therapy from the view point of angiogenesis and neuroregeneration in DPN. This is the first report demonstrating the therapeutic effects of NCL cell transplantation on DPN.

2. Research Results

iPS cells were induced to differentiate into neural cells by stromal cell-derived inducing activity (SDIA) and subsequently supplemented with bone morphogenetic protein 4 to promote a differentiation of NC lineage. After the induction, p75 neurotrophin receptor positive NCL cells were purified using magnetic-activated cell sorting. Sorted NCL cells differentiated to peripheral neurons, glial cells and smooth muscle cells by additional SDIA. NCL cells were transplanted into hind limb skeletal muscles of 16-week streptozotocin-diabetic mice. Nerve conduction velocity, current perception threshold, intraepidermal nerve fiber density, sensitivity to thermal stimuli, sciatic nerve blood flow, plantar skin blood flow and capillary number–to–muscle fiber ratio were evaluated. Four-week after the transplantation, transplanted cells engrafted with producing growth factors; nerve growth factor, neurotrophin 3, vascular endothelial growth factor

and basic fibroblast growth factor. It was also confirmed that some engrafted cells differentiated into vascular smooth muscle cells or Schwann cell like cells at each intrinsic site. The transplantation improved the impaired nerve and vascular functions. These results suggest that transplantation of NCL cells derived from iPS cells could have therapeutic effects on DPN through paracrine actions of growth factors and differentiation into Schwann cell like cells and vascular smooth muscle cells.

3. Research Summary and Future Perspectives

We first demonstrated the beneficial effects of transplantation of NCL cells on DPN by using GFP-positive iPS cells. NCL cells may have practical advantages for regenerative medicine, such as their easy accessibility and differentiation potential. The advantage of NCL cells for transplantation therapy actions is that they can exert the dual actions of cell replacement and supplying growth factors both of which are required in the treatment of DPN. Although further studies designed to reveal additional useful aspects of NCL cell transplantation on DPN might be required, the transplantation of NCL cells appears to be a promising therapeutic strategy for DPN.

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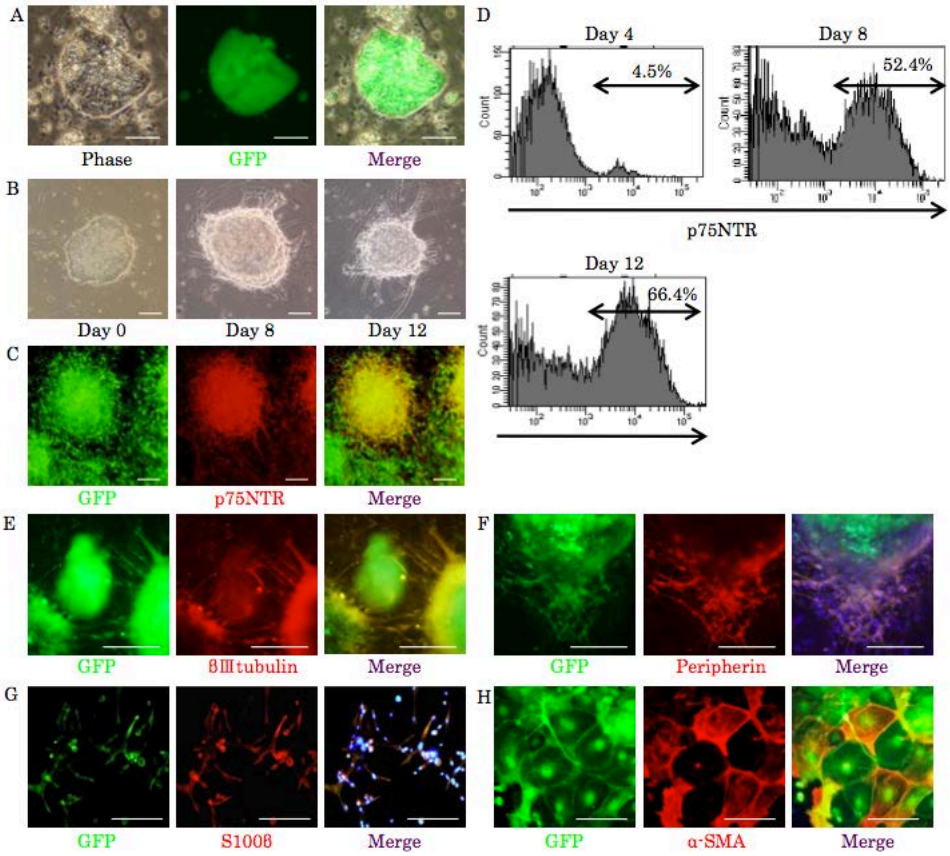

Figure. Induction of NCL cells from aged mouse iPS cells.

(A) GFP-positive iPS cell. (B) Phase-contrast photographs of mouse iPS cell colonies co-cultured with PA6 cells. Neurite-like processes were extended from the colonies over time. (C) Expression of p75NTR (red), a cell surface marker of neural crest cells, at day 12. (D) FACS analysis of differentiated cells was conducted after 4, 8, 12 days of co-culture. Cells were labeled with APC-conjugated antibodies for p75NTR. Scale bars: 100μm.

(E-H) Ability of the p75NTR positive cells to differentiate into neural crest derivatives *in vitro*. After the further induction of purified p75NTR positive cells on PA6 cells, a number of differentiated cells expressed βⅢtubulin and peripherin (red) and extended neurite-like processes (E, F). Many of induced cells were immunostained with anti-S100β antibody (red) (G) anti-α-smooth muscle actin antibody (red) (H). Nuclei were stained with DAPI (blue). Scale bars: 100 μm. αSMA; α-smooth muscle actin.