The Rotary Cell Culture System increases NTRK3 Expression and Promotes Neuronal Differentiation and Migratory Ability of Neural Stem Cells Cultured on Collagen Sponge

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Research

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Abstract

Background Recently, neural stem cell (NSC) therapy has shown promise for the treatment of many neurological diseases. Enhancing the quality of implanted cells and improving therapeutic efficacy are currently research hotspots. It has been reported that collagen sponge material provided sufficient room for cell growth in all directions, promoted the absorption of nutrients and removal of wastes. And also, the Rotary Cell Culture System (RCCS), which mimics the microgravity environment, can be used to culture cells for tissue engineering.

Materials and methods We performed the mRNA and miRNA sequencing to elucidate the regulatory mechanism of NSCs cultured on the collagen sponge in RCCS system. The luciferase assay and Western blot revealed a direct regulatory role between let-7i-5p and NTRK3. And then, the neural differentiation maker Tuj1 and Map2 were detected by immunofluorescence staining. In the meantime, the migratory ability of NSCs was detected both in vitro and in spinal cord injury animals.

Results In this study, we demonstrated that the expression of neurotrophic receptor tyrosine kinase 3 (NTRK3; also called TrkC) was elevated in NSCs cultured on collagen sponge in the RCCS system. Furthermore, increased NTRK3 expression was regulated by the downregulation of let-7i-5p. Compared to traditionally cultured NSCs, the NSCs cultured on collagen sponge in the RCCS system exhibited better neuronal differentiation and migratory ability, especially in the presence of NT-3.

Conclusions As the biological properties and quality of transplanted cells are critical for therapeutic success, the RCCS system combined with collagen sponge culture system show promise for applications in clinical practice in the future.

Full Text

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Figures

(A)

(B)

Figure 1

(A) A flowchart of the experiment. (B) Scanning electron microscopy revealed the morphology of the collagen sponge scaffold (left) and 3D cultured NSCs (middle); fluorescein diacetate staining of the 3D cultured NSCs (right).

Figure 2

The differentiation and migratory ability of 3D cultured NSCs in all groups detected by immunofluorescence staining (A) and trans-well migration array (B).

Figure 3

The interaction between let-7i-5p and NTRK3. (A) Bioinformatics analysis predicted a regulatory role of let-7i-5p on NTRK3. (B) Real-time PCR indicates let-7i-5p and NTRK3 expression in the RCCS and control groups. (C) The luciferase reporter array indicated an interaction between 3'-UTR of NTRK3 and the seed sequence of let-7i-5p. (D) Western blot indicated that NTRK3 protein levels are affected by let-7i-5p mimics and inhibitors.

Figure 4

Immunofluorescence staining indicated an effect of let-7i-5p and NTRK3 on NSC differentiation when the differentiation medium contained NT3; (A) Tuj1 (Neuronal Class III β -Tubulin), (B) Map2 (microtubule associated protein 2), (C) GFAP (glial fibrillary acidic protein).

Figure 5

The transwell migration assay indicated an effect of let-7i-5p and NTRK3 on NSC migration in the presence of NT3.

Figure 6

The transplantation of NSCs in rats with spinal cord injury. (A) Spinal cord injuries (with lengths of 3 mm) were inflicted in rats (left). Functional collagen sponge scaffolds with or without cells were precisely placed into the injury site (middle). Samples of the spinal cord tissue following perfused 4% paraformaldehyde fixation (right). (B) GFAP immunostaining in all groups. The green signal represents the GFP-labelled transplanted NSCs. (C) Enlarged images of the migrated GFP+ cells in the RCCS and RCCS+NT-3 groups.