A new function for radial glial cells in white matter formation and implications for regeneration

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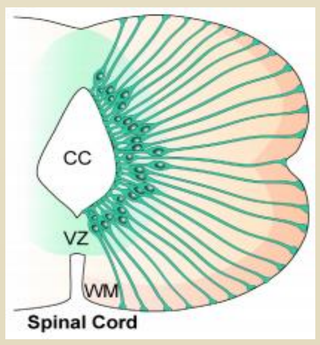
POSTER OVERVIEW

This study shows that the growth of sensory and motor tracts though the spinal cord is tightly regulated by radial glial cells, a transient neural stem cell population.

This new function for radial glial cells is especially significant for developmental defects affecting motor and sensory systems and raises multiple therapeutic opportunities for radial glia in axonal regeneration following disease or injury.

INTRODUCTION

Radial glia are neural stem cells present in the CNS during development that play multiple roles in the correct development of the brain.



In the spinal cord radial glia exist transiently during development. They exhibit a cell body in the ventricular zone (VZ) and extend from the central canal (CC) to the pial surface

They function as guidance conduits for migrating neurons and as multipotent stem cells, generating both neurons and glia, thereby populating the CNS (Barry et al, 2013).

Radial glial processes extend through all growing axon tracts during development; however, the relationships between radial glial cells and the developing white matter are largely unknown, especially in the spinal cord.

FINDINGS

We show that radial glia are highly organised in the developing spinal cord during axonogenesis.

Radial glia act as structural scaffolds supporting the formation of dorsal axon tracts, which carry sensory information to the brain.

3. We have identified FORSE-1 (forebrain-surface embryonic) as a candidate signalling cue that may mediate these guidance functions.

MATERIALS AND METHODS

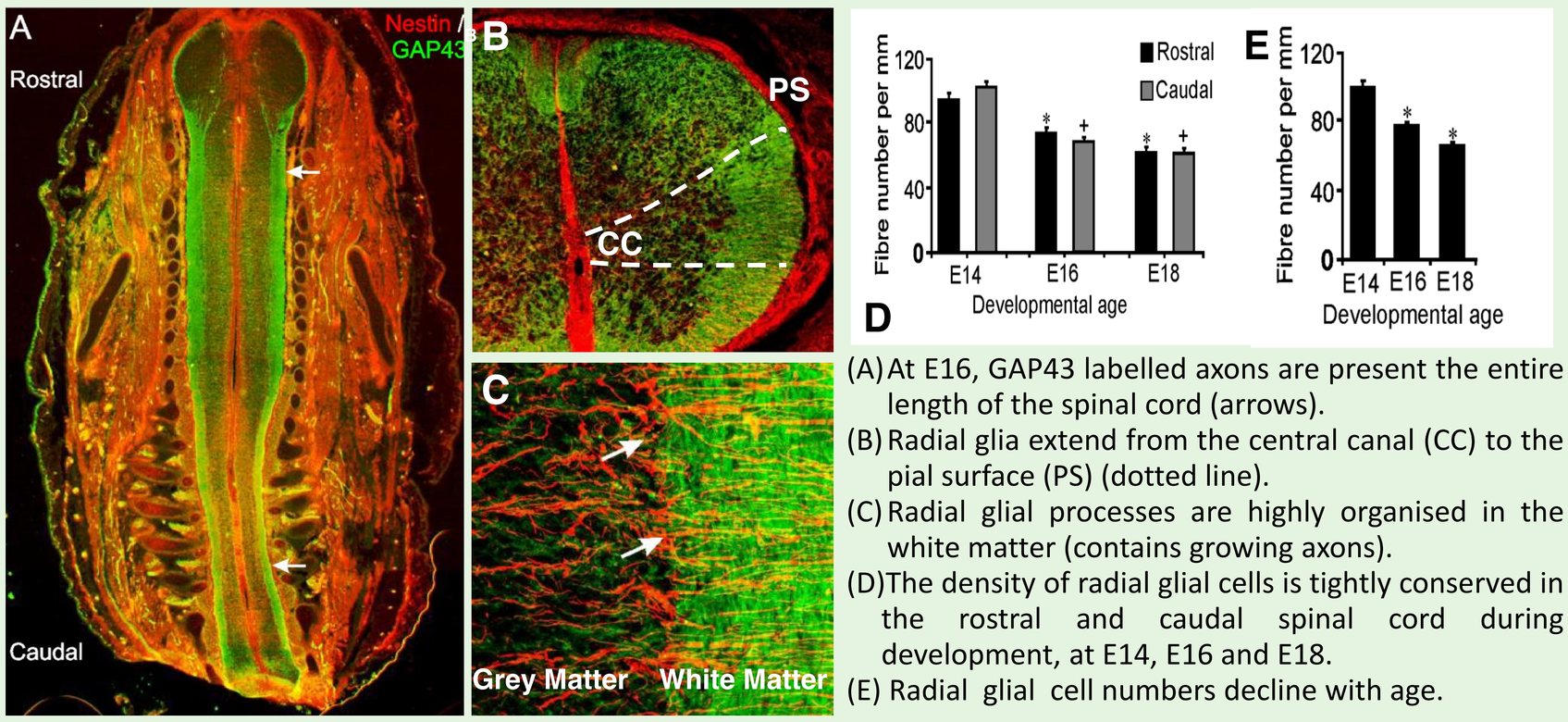
15 µm cryosections of mouse and rat embryos ages E14, E16 and E18 were cut from the cervical, thoracic and lumbar regions of the spinal cord. These were washed PBS and blocked in 20% NGS and 0.2% Triton-X 100.

Primary antibodies used were
- Nestin (1:100 DSHB) - radial glial cells
- Vimentin (1:200 Sigma)- radial gial cells
- FORSE-1 (1:100 DSHB) - radial glial cells
- GAP43 (1:200 Chemicon) – axon growth cones

DAPI (1:1000) or propidium iodide (PI) (1: 20000) were used to label cell nuclei.

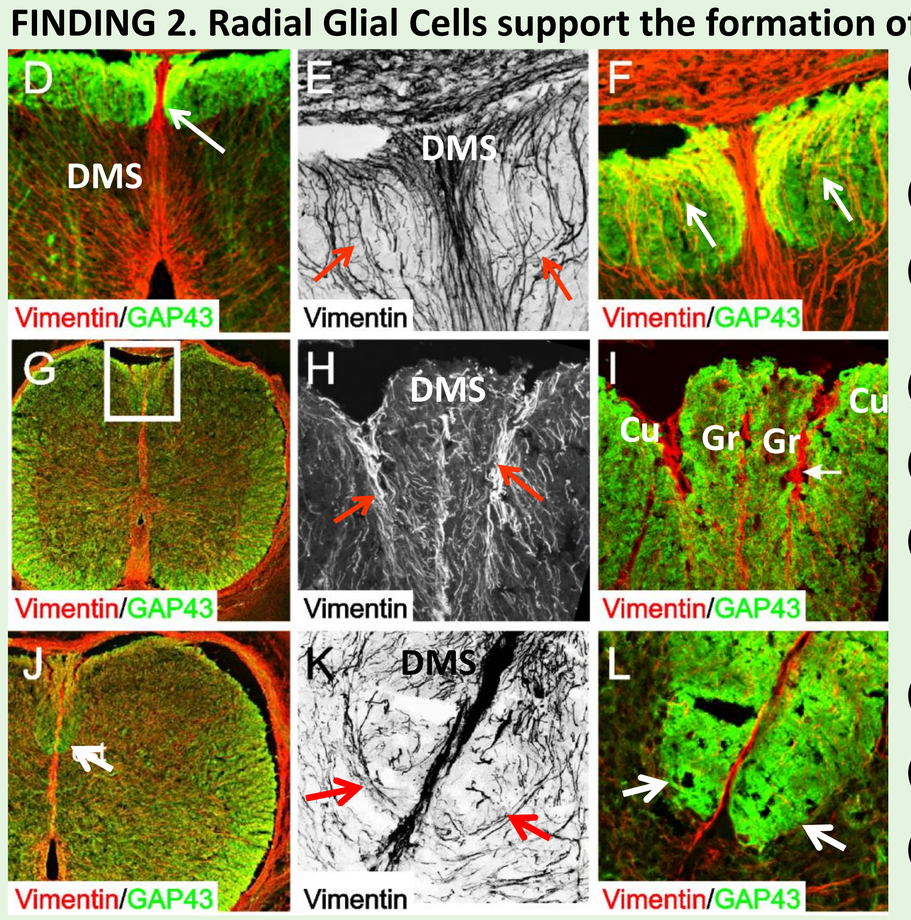
Images was acquired on an Olympus IX81 fluorescent microscope using Cell Sens imaging software.

FINDING 1. Radial glial cells are highly organised as they pass through the growing white matter



(A) At E16, GAP43 labelled axons are present the entire length of the spinal cord (arrows).
(B) Radial glia extend from the central canal (CC) to the pial surface (PS) (dotted line).
(C) Radial glial processes are highly organised in the white matter (contains growing axons).
(D) The density of radial glial cells is tightly conserved in the rostral and caudal spinal cord during development, at E14, E16 and E18.
(E) Radial glial cell numbers decline with age.

FINDING 2. Radial Glial Cells support the formation of sensory axon tracts

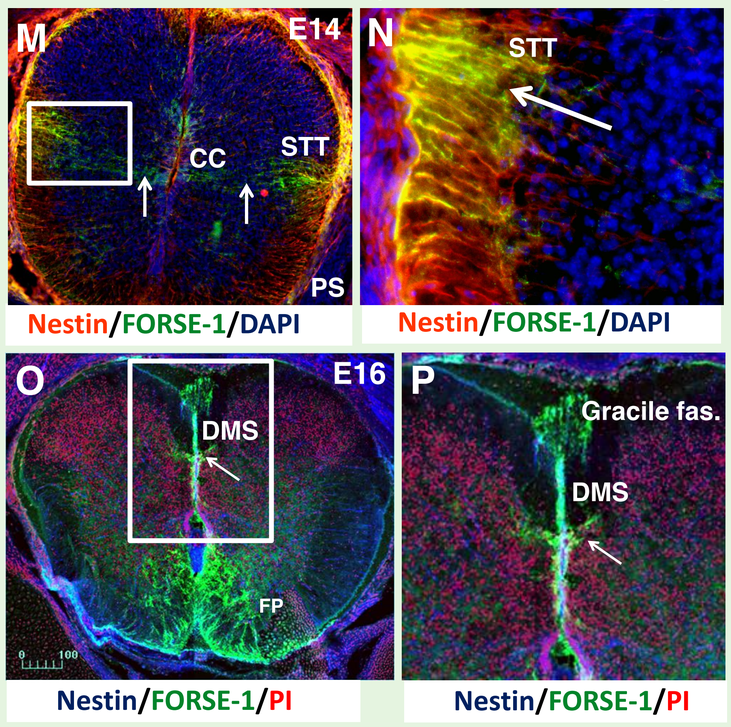


(D) At E16, radial glia form the dorsal medial septum (DMS). This divides the emerging GAP43 labelled axons of the dorsal column bilaterally (arrow).
(E) Fibres from the DMS form a supportive scaffold network (arrows).
(F) The early arriving axons forming the dorsal column grow within this DMS scaffold (arrows).

(G) At E17, Radial glial processes separate the emerging cuneate and gracilis fasciculi (box).
(H) Highly arranged bands of radial glia stem from the DMS and grow to the pial surface (arrows).
(I) These bands of radial glia separate the growing cuneate (Cu) and gracile (Gr) axon tracts from each other.

(J) At E18, the ventral portion of the dorsal fasciculi extends into the grey matter (arrow).
(K) Radial glial cell processes form a sling that extends from the DMS (arrows).
(L) The ventral portion of the dorsal column grows inside this sling (arrows).

FINDING 3. FORSE-1 is present on radial glial processes in regions where sensory tracts are growing



M. At E14, radial glial extend from the central canal (CC) to the pial surface (PS). FORSE-1 is present on radial glial cell processes in the lateral white and grey matter of the spinal cord (arrows).

N. FORSE-1 is more intensely expressed on radial glial cells in the region of the growing spinothalamic tract (STT) (arrows).

O. At E16, radial glial cells still extend from the central canal to the pial surface, however FORSE-1 is now expressed on the DMS, the glial scaffold (as in K), and the floor plate (FP).

P. FORSE-1 is expressed on radial glial cells forming the glial scaffold surrounding the ventral dorsal column. It is also expressed on the gracile fasciculus, but not the cuneate fasciculus.

CONCLUSION

The growth of motor and sensory axon tracts through the spinal cord occurs through organised radial glial processes in the ventral, lateral and dorsal spinal cord. They appear to form channels through axons grow. These channels disappear after axon tracts have matured.

Radial glial processes compartmentalise growing sensory axon tracts in the dorsal spinal cord. They form scaffolds and permissive corridors through which axons are directed and partitioned from the surrounding grey matter.

FORSE-1 is present on radial glial cell in regions of the spinal cord where sensory axon tracts grow, including the spinothalamic tract and the cuneate and gracile fasciculi, where it is likely acting as a guidance cue.

This new function for radial glial cells is especially significant for developmental defects affecting motor and sensory systems and raises multiple therapeutic opportunities for radial glia in axonal regeneration following disease or injury.