**Supplementary Materials**

**Celsr2-mediated morphological polarization and functional phenotype of reactive astrocytes in neural repair**

Aimei Liu1, 2 #, Lingtai Yu1 #, Xuejun Li1 #, Kejiao Zhang1, Wei Zhang1, Kwok-Fai So1, 2, 3, 4, 5, Fadel Tissir 6, 7, Yibo Qu1, 4, 5 \*, Libing Zhou1, 2, 3, 4, 5 \*

\*Corresponding authors. Emails: tlibingzh@jnu.edu.cn and tquyibo@jnu.edu.cn

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**Other Supplementary Materials for this manuscript include the following:**

Movies S1 to S2

**Magnetic resonance imaging（MRI）**

Two months after SCI, mice were anesthetized (1.0-2.0% isoflurane) and placed on an acrylic bed with a heating equipment, breathing and temperature monitor and an electrocardiogram (ECG) probe for MRI imaging. Animals were Sagittal and axial T2-weighted images (T2WI) and diffusion tensor imaging (DTI) were performed with a 9.4 T bore scanner (Bruker Biospec, Ettlingen, Germany), using a single-channel surface coil (diameter of 20 mm). DTI images were obtained using EPI-DTI sequences with an ECG-gated standard diffusion-weighted spin-echo pulse sequence in order to reduce motion artifacts, with the following parameters: repetition/echo time= 4000/22 ms, flip angle= 90°, 30 noncollinear gradient directions, 5 A0 images, b = 1000 sec/mm2, 6 averages, 1mm thickness, FOV = 30 × 30 mm, matrix dimension = 150 × 150(*34*). DTI studio was used for Fractional anisotropy (FA), Radial diffusion (RD) calculations. Sizes of spinal cord lesions were calculated using Image J.

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**Fig. S1. Celsr2 expression in some oligodendrocytes and active microglia, but not in quiescent microglia.**

A, B: Immunostaining of spinal transverse sections from 1-month-old *Celsr2LacZ* transgenic mice. *β*-gal signal is present in a subpopulation of Olig2-positive oligodendrocytes (A), but not in Iba1-postive microglia (B).

C, D: Seven days after SCI, double immunostaining shows that *β*-gal is positive in some Iba1-postive reactive microglia (C) and CD68-positive macrophages (D).

White and red arrows indicate cells positive and negative for *β*-gal signal, respectively.

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**Fig. S2. *Celsr2* negatively regulates astrocyte migration but not proliferation *in vitro*.**

A, B: In the transwell assay, Hoechst staining shows an increase of migrating astrocytes through the membranes in the mutant (B; n=20 objective fields in each group).

C, D: EdU labelling indicates a comparable proliferation of astrocytes in both groups (D; n=20 fields in each group).

\*\*\*\*, *P*<0.0001; ns, not significant; Student’s *t*-test.

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**Fig. S3.** **MRI discloses morphological improvement of injured spinal cords in *Celsr2-/-* mice.**

A: MRI scanning of live animals shows lesion areas 2 months after SCI (A; arrows). In transverse sections, lesion cavities are outlined by dotted circles.

B-D: Statistical analysis shows a significant increase of FA values (B) at 3 mm, 2 mm, -2 mm and -3 mm, a significant decrease of RD values (C) at 1 mm, 0 mm, -1 mm and -2 mm, and smaller lesion cavity (D) at 1 mm, 0 mm and -1 mmin *Celsr2-/-* animals. \*, *P*<0.05; \*\*, *P*<0.01; Two-way repeated ANOVA with Bonferroni’s *post-hoc* correction; n=5 in each group.

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**Fig. S4. Inhibitor administration downregulates Cdc42 and Rac1 activity in *Celsr2-/-* spinal samples after SCI.**

Spinal samples (T9-T11 segments) are collected from wildtype (control) and *Celsr2*-*/-* mice 7 days after SCI. Western blots are performed using whole protein extracts or the proteins eluted from GST-bound beads (A). ‘*Celsr2-/-*+inhibitors’ indicates the samples from animals with 7-day inhibitor treatment. Statistical analysis shows a significant increase of total Cdc42 proteins (B), GTP-bound Cdc42 (C), total Rac1 proteins (D) and GTP-bound Rac1 (E) in the *Celsr2*-*/-* compared to the control and *Celsr2-/-*+inhibitors respectively. \*, *P*<0.05; \*\*, *P*<0.01; \*\*\*, *P*<0.001; One-way ANOVA analysis of variance with Tukey’s multiple comparison tests, n=3 animals/group; GAPDH, reference control.

**Table S1. Primers for RT-qPCR**

|  |  |  |
| --- | --- | --- |
| **Genes** | **Forward Sequence** | **Reverse Sequence** |
| *B3gnt5* | CGTGGGGCAATGAGAACTAT | CCCAGCTGAACTGAAGAAGG |
| *Celsr2* | CACGATGGCCTGAGGGTTT | CCTTGTGGAGAAAGGTGTCCT |
| *C3* | CGCAACGAACAGGTGGAGATCA | CTGGAAGTAGCGATTCTTGGCG |
| *Clcf1* | CTTCAATCCTCCTCGACTGG | TACGTCGGAGTTCAGCTGTG |
| *GAPDH* | CCAATGTGTCCGTCGTGGATCT | GTTGAAGTCGCAGGAGACAACC |
| *Ptx3* | AACAAGCTCTGTTGCCCATT | TCCCAAATGGAACATTGGAT |
| *Serping1* | ACAGCCCCCTCTGAATTCTT | GGATGCTCTCCAAGTTGCTC |
| *Tm4sf1* | GCCCAAGCATATTGTGGAGT | AGGGTAGGATGTGGCACAAG |

**Table S2 (extended to Fig. 1D).** Protrusion length of reactive astrocytes in the wound scratch assay.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Control | *Celsr2-/-* | P value |
| Experiment-1 | 83.51 ± 5.79 (41) | 102.8 ± 5.79 (36 ) | <0.05 |
| Experiment-2 | 71.32 ± 4.88 (34) | 98.67 ± 5.67 (45) | <0.0001 |
| Experiment-3 | 78.78 ± 7.59 (27) | 115.6 ± 9.86 (25) | <0.01 |

Data presented as mean ± SEM in μm; values in brackets: cell numbers for analysis in each experiment.

**Table S3 (extended to Fig. 1F).** Reactive astrocyte coverage in wound scratch assay.

|  |  |  |  |
| --- | --- | --- | --- |
| Experiment | Control | *Celsr2-/-* | P value |
| Experiment-1 | 72.99 ± 4.25 (3) | 96.43 ± 4.13 (3) | <0.05 |
| Experiment-2 | 62.16 ± 3.65 (3) | 81.63 ± 4.46 (3) | <0.05 |
| Experiment-3 | 43.42 ± 2.49 (6) | 63.16 ± 3.67 (3) | <0.01 |
| Experiment-4 | 51.06 ± 3.05 (3) | 73.17 ± 3.75 (3) | <0.05 |

Data presented as mean ± SEM (%): coverage percentage of migrating astrocytes to the lesion area; values in brackets: numbers of coverslips (one scratch/coverslip) for analysis in each experiment.

**Table S4 (extended to Fig. 3D).** Astrocyte protrusion length of cultured cells treated by Cdc42 inhibitor (ML141) in wound scratch assay.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Sham | Control | *Celsr2-/-* |
| Experiment-1 | 74.95 ± 3.32 (34) | 60.55 ± 4.01 (33)\*\* | 66.08 ± 2.90 (33)\*, ns |
| Experiment-2 | 77.62 ± 4.59 (35) | 64.68 ± 3.96 (36) \* | 65.17 ± 3.40 (35) \*, ns |
| Experiment-3 | 78.97 ± 4.37 (47) | 62.11 ± 2.36 (47) \*\* | 65.80 ± 3.25 (48) \*, ns |

The control and *Celsr2-/-* groups: cultured astrocytes from wildtype and *Celsr2-/-* mice treated by ML141; the sham: cultured astrocytes from wildtype without inhibitor treatment. Data presented as mean ± SEM in μm. \* and \*\*: *P*<0.05 and 0.01 indicating the comparison to the sham; ns: not significant, indicating the comparison between the control and the *Celsr2-/-*. Values in brackets: cell numbers for analysis in each experiment.

**Table S5 (extended to Fig. 3G).** Astrocyte protrusion length of cultured cells treated by Rac1 inhibitor (NSC23768) in wound scratch assay.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Sham | Control | *Celsr2-/-* |
| Experiment-1 | 78.11 ± 4.74 (33) | 54.79 ± 2.55 (33) \*\*\* | 60.83 ± 4.38 (33) \*\*, ns |
| Experiment-2 | 74.64 ± 3.19 (41) | 58.97 ± 3.42 (41) \*\* | 63.90 ± 3.42 (41) \*, ns |
| Experiment-3 | 79.50 ± 4.70 (42) | 59.84 ± 3.96 (42) \*\* | 65.11 ± 3.51 (42) \*, ns |

The control and *Celsr2-/-* groups: cultured astrocytes from wildtype and *Celsr2-/-* mice treated by NSC23768; the sham: cultured astrocytes from wildtype without inhibitor treatment. Data presented as mean ± SEM in μm. \*, \*\*, \*\*\*: *P*<0.05, 0.01, 0.001, indicating the comparison to the sham; ns: not significant, indicating the comparison between the control and the *Celsr2-/-*. Values in brackets: cell numbers for analysis in each experiment.

**Table S6 (extended to Fig. 4D).** Protrusion length of reactive astrocytes surrounding lesion border in wildtype (control) and *Celsr2-/-* mice after SCI.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Control |  | *Celsr2-/-* |
| Mouse 1 | 72.87 ± 1.65 (137) | Mouse 1 | 97.71 ± 2.48 (129) |
| Mouse 2 | 79.91 ± 2.31 (100) | Mouse 2 | 133.1 ± 4.08 (100) |
| Mouse 3 | 71.51 ± 2.07 (100) | Mouse 3 | 127.8 ± 3.28 (100) |
| Mouse 4 | 63.67 ± 1.25 (100) | Mouse 4 | 118.1 ± 2.34 (100) |
| Mouse 5 | 83.94 ± 2.51 (100) | Mouse 5 | 109.8 ± 3.77 (100) |

Data presented as mean ± SEM in μm. Values in brackets: cell numbers for analysis in each mouse.

**Movie S1.**

Calcium imaging in control samples.

**Movie S2.**

Calcium imaging in *Celsr2-/-* samples.