Astrocytic YAP Protects the Optic Nerve and Retina in an Experimental Autoimmune Encephalomyelitis Model Through TGF- β Signaling

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Research

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Abstract

Background

Optic neuritis, inflammation of the optic nerve (ON), is one of the main symptoms in multiple sclerosis (MS) and leads to visual disability. Astrocytes are pivotal regulators of neuroinflammation in MS. However, it remains unclear that the detailed roles and mechanisms of astrocytes in the neuroinflammation and demyelination in optic neuritis of MS.

Methods

To assess the role of YAP in ON and retina in response to experimental autoimmune encephalomyelitis (EAE), mice that conditionally knockout (CKO) YAP in astrocytes, namely YAP GFAP-CKO mice, were successfully generated. Immunostaining, Nissl staining, Hematoxylin-Eosin (HE) staining, TUNEL staining, luxol fast blue (LFB) staining, electron microscopy (EM), qRT-PCR and gene set enrichment analysis (GSEA) and gene set variation analysis (GSVA) by RNA sequencing were used to examine the roles of YAP pathway in EAE based on these conditional knockout mice. Inhibitors including SRI-011381 [an agonist of transforming growth factor- β (TGF- β) pathway] and XMU-MP-1 (an inhibitor of Hippo kinase MST1/2 to activate YAP) were used to further explore the molecular mechanism of YAP in ON and retina of EAE mice. Additionally, microglia and retinal ganglion cells (RGCs) counts, and demyelination and astrocytes were assessed by immunohistological staining.

Results

We found that yes-associated protein (YAP) was significantly upregulated and activated in the astrocytes of ON in EAE. Conditional knockout YAP in astrocytes caused more severe inflammatory infiltration and demyelination in ON, and damage of the RGCs in EAE mice. Moreover, YAP deletion in astrocytes promoted the activation of astrocytes and microglia, but inhibited the proliferation of astrocytes of ON in EAE mice. Mechanically, TGF- β signaling pathway was significantly down-regulated after YAP deletion in astrocytes. Additionally, both qPCR and immunofluorescence assays confirmed the reduction of TGF- β 1 in YAP knockout ON astrocytes of EAE mice. Interestingly, SRI-011381 partially rescued the deficits in ON and retina of YAP knockout EAE mice. Finally, activation of YAP pathway relieved the neuroinflammation and demyelination in optic neuritis of EAE mice.

Conclusions

These results suggest astrocytic YAP may prevent the neuroinflammatory infiltration and demyelination through upregulation of TGF- β signaling and provide targets for the development of therapeutic strategies tailored for optic neuritis in MS.

Full Text

Figures

Figure 1

Figure 1

YAP is upregulated and activated in astrocytes of ON of EAE mice. (A) Immunohistochemistry detected the YAP expression in the ON of control and EAE mice. (B) Quantitative analysis of the number of YAP⁺ cells per mm² as shown in (A) (n=8, per group). (C) Double immunostaining of YAP (green) and GFAP (red) in the ON of control and EAE mice. (D) Quantitative analysis of the number of nuclear YAP⁺ astrocytes per mm² as shown in (C) (n=6, per group). (E) Double immunostaining of YAP (green) and Iba1 (red) in the ON of control and EAE mice. Images of selected regions (white squares) were shown at higher magnification. Data were mean ± SEM, Student's t-test, compared with control mice, ***P<0.001. Scale bars, 20 μm.

Figure 2

Figure 2

Ablation of YAP in astrocytes exacerbates the demyelination in ON of EAE mice. (A) LFB staining in the ON obtained from control, YAP^{fl/fl} and YAP^{GFAP-CKO} EAE mice. (B) Quantitative analysis of the demyelination score as shown in (A) (n=8, per group). (C) Immunostaining of MBP (green) in the ON obtained from control, YAP^{fl/fl} and YAP^{GFAP-CKO} EAE mice. (D) Quantitative analysis of MBP intensity as shown in (C) (n=12, per group). (E) The longitudinal section of the ON obtained from YAP^{fl/fl} and

YAPGFAP-CKO EAE mice under transmission electron microscopy consists of the myelinated axons and myelin sheath. (F) Quantitative analysis of the thickness of myelin sheath as shown in (E) (n=25, per group). Images of selected regions (white squares) were shown at higher magnification. Data were mean $\pm$ SEM, two-way ANOVA with Bonferroni's post-tests, compared with control mice, *P<0.05, ***P<0.001. Scale bars, 20 μ m.

Figure 3

Ablation of YAP in astrocytes exacerbates severe inflammatory infiltration in ON of EAE mice. (A) HE staining of the ON obtained from control, YAPf/f and YAPGFAP-CKO EAE mice. (B) Quantitative analysis of the density of inflammatory cells as shown in (A) (n=10, per group). (C) Immunostaining of CD45 (red) in the ON obtained from control mice, YAPf/f and YAPGFAP-CKO EAE mice. (D) Quantitative analysis of the number of CD45+ cells per mm² as shown in (C) (n=9, per group). (E) Double immunostaining of Iba1 (green) and GFAP (red) in the ON obtained from control, YAPf/f and YAPGFAP-CKO EAE mice. (F) Quantitative analysis of GFAP intensity as shown in (E) (n=9, per group). (G) Quantitative analysis of the number of Iba1+ cells per mm² as shown in (E) (n=12, per group). (H) Immunostaining of CD206 (green) in the ON obtained from control, YAPf/f and YAPGFAP-CKO EAE mice. (I) Quantitative analysis of the number of CD206+ cells per mm² as shown in (H) (n=12, per group).

(I) Quantitative analysis of the number of CD206+ cells per mm² as shown in (H) (n=10, per group). Images of selected regions (white squares) were shown at higher magnification. Data were mean $\pm$ SEM, two-way ANOVA with Bonferroni's post-tests, compared with control mice, *P<0.05, **P<0.01, ***P<0.001. Scale bars, 20 μ m.

Figure 4

Ablation of YAP affect the proliferation of astrocytes and microglia in ON of EAE mice. (A-B) Double immunostaining of Ki67 (green) and GFAP (red) (A) or PH3 (green) and GFAP (red) (B) in the ON of control, YAP^{f/f} and YAP^{GFAP-CKO} EAE mice. (C-D) analysis of the percentage of Ki67+ (C) or PH3+ (D) cells over total astrocytes as shown in (A-B) (n=7, per group). (E-F) Double immunostaining of Ki67 (green) and Iba1 (red) (E), or PH3 (green) and Iba1 (red) (F) in the ON of control, YAP^{f/f} and YAP^{GFAP-CKO} EAE mice. (G-H) Quantitative analysis of the percentage of Ki67+ (G) or PH3+ (H) cells over total microglia as shown in (E-F) (n=10, per group). Images of selected regions (white squares) were shown at higher magnification. Data were mean $\pm$ SEM, two-way ANOVA with Bonferroni's post-tests, compared with control mice, *P<0.05, **P<0.01, ***P<0.001. Scale bars, 20 μ m.

Figure 5

Figure 5

Ablation of YAP in astrocytes exacerbates inflammation and apoptosis in retina of EAE mice. (A, C, E) Immunostaining of Iba1 (green) (A), GFAP (red) (C) or Vimentin (cyan) (E) in the retina of control, YAP^{f/f} and YAPGFAP-CKO EAE mice. (B) Quantitative analysis of the number of Iba1⁺ cells per mm² as shown in (A) (n=7, per group). (D, F) Quantitative analysis of GFAP intensity (D) or Vimentin intensity (F) as shown in (C, E) (n=8, per group). (G) Representative images of Nissl staining in the retina of control, YAP^{f/f} and YAPGFAP-CKO EAE mice. (H) Quantitative analysis of the number of Nissl⁺ cells per mm² as shown in (G) (n=11, per group). (I, K) Immunostaining of RBPMS (green) (I) or NeuN (green) (K) in the retina of control, YAP^{f/f} and YAPGFAP-CKO EAE mice. (J, L) Quantitative analysis of the number of RBPMS⁺ cells (J) or NeuN⁺ cells (L) per mm² as shown in (I, K) (n=7, per group). (M) Immunostaining analysis of cell apoptosis by TUNEL staining in the retina of control, YAP^{f/f} and YAPGFAP-CKO EAE mice. (N) Quantitative analysis of the number of TUNEL⁺ cells per mm² as shown in (M) (n=9, per group). Data were mean ± SEM, two-way ANOVA with Bonferroni's post-tests, compared with control mice, *P<0.05, **P<0.01, ***P<0.001. Scale bars, 20 μm.

Figure 6

Figure 6

GSEA and GSVA in YAP+/+ vs YAP-/- astrocytes. (A) The 11 down-regulated gene set with statistically significant (FDR < 0.25) in YAPGFAP-CKO group. (B) The enrichment plot of TGF- β signaling gene sets. (C) The clustering heatmap of 49 genes in TGF- β signaling gene sets. Red, blue and white respectively represent high expression level, low expression level and no expression difference among the genes. (D) GSVA-derived clustering heatmap of differentially expressed gene sets. Red, blue and white respectively represent high expression level, low expression level and no expression difference among the genes.

Figure 7

Figure 7

Activation of TGF- β signaling reduces inflammatory infiltration and demyelination in the ON of EAE mice. (A) Immunostaining of TGF- β 1 (green) and GFAP (red) in the ON of control, SRI-011381-treated YAP^{f/f} and YAP^{GFAP-CKO} EAE mice. Images of selected regions (white squares) were shown at higher magnification. (B) Quantitative analysis of the number of TGF- β 1+ astrocytes per mm² as shown in (A) (n=5, per group). (C) LFB staining in the ON of control, SRI-011381-treated YAP^{f/f} and YAP^{GFAP-CKO} EAE mice. (D) Quantitative analysis of the demyelination score of control, SRI-011381-treated YAP^{f/f} and YAP^{GFAP-CKO} EAE mice as shown in (C) (n=7, per group). (E, G, I, K) Immunostaining of MBP (green) (E), Iba1 (green) (G), GFAP (green) (I) or CD45 (green) (K) in the ON of control, SRI-011381-treated YAP^{f/f} and YAP^{GFAP-CKO} EAE mice. (F, J) Quantitative analysis of MBP (F) or GFAP (J) intensity as shown in (E, I) (n=6, per group). (H, L) Quantitative analysis of the number of Iba1+ cells (H) or CD45+ cells (L) per mm² as shown in (G, K) (n=7, per group). Images of selected regions (white squares) were shown at higher magnification. Data were mean $\pm$ SEM, two-way ANOVA with Bonferroni's post-tests, compared with control mice, **P<0.01, ***P<0.001. Scale bars, 20 μ m.

Figure 8

Figure 8

XMU-MP-1 reduces inflammation and demyelination of ON, and the loss of RGCs in EAE mice. (A) LFB staining of the ON obtained from control and XMU-MP-1-treated EAE mice. (B) Quantitative analysis of the demyelination score of control and XMU-MP-1-treated EAE mice as shown in (A) (n=7, per group). (C, E, G, I) Immunostaining of MBP (green) (C), Iba1 (green) (E), GFAP (green) (G) or CD45 (green) (I) in the ON of control and XMU-MP-1-treated EAE mice. (D, H) Quantitative analysis of MBP intensity (D) or GFAP

intensity (H) as shown in (C, G) (n=7, per group). (F, J) Quantitative analysis of the number of Iba1+ cells (F) or CD45+ cells (J) per mm² as shown in (E, I) (n=6, per group). (K, M, O) Immunostaining of Iba1 (green) (K), GFAP (green) (M) or NeuN (green) (O) in the retina of control and XMU-UP-1-treated EAE mice. (L, P) Quantitative analysis of the number of Iba1+ cells (L) or NeuN+ cells (P) per mm² as shown in (K, O) (n=5, per group). (N) Quantitative analysis of GFAP intensity as shown in (M) (n=7, per group). Images of selected regions (white squares) were shown at higher magnification. Data were mean $\pm$ SEM, Student's t-test, compared with control mice, *P<0.05, **P<0.01, ***P<0.001. Scale bars, 20 μ m.

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