Molecular mechanism of microRNA-21 on regulating astrocyte activation and the effect of microRNA-21 on axon regeneration after optic nerve injury

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Abstract

Background:

It is difficult to regenerate and repair after optic nerve injury. RGCs died due to secondary adverse axoplasmic transport disorder, which eventually led to irreversible visual dysfunction and seriously affected the quality of life of patients. In the case of optic nerve injury, the regeneration process is regulated by the damage microenvironment, and the excessive activation of optic nerve astrocytes is an important factor causing the disorder of optic nerve regeneration. Müller cell is a special type of astrocyte in retina. Recent studies have found that microRNA-21 (miR-21) plays an important regulatory role in the activation of astrocytes after nerve injury. However, the regulatory mechanism of miR-21 on the activation of astrocytes and retinal Müller cells in the optic nerve and its effect on axon regeneration and visual function after optic nerve injury are still unclear.

Objective:

1. To investigate the molecular mechanism of miR-21 regulating the activation of optic nerve astrocytes in vitro.

2. To investigate the molecular mechanism of miR-21 regulating the activation of astrocytes in the context of optic nerve injury and its influence on axon regeneration and visual evoked potential.

3. To investigate the regulatory mechanism of miR-21 on the activation of retinal Müller cell in the context of optic nerve injury and its influence on RGC survival and visual function.

Methods:

1. The activated astrocytes were transfected with miR-21 mimic/inhibitor and related inhibitors to investigate the molecular mechanism of miR-21 regulating the activation of optic nerve astrocytes in vitro.

2. A rat model of standardized optic nerve injury was established. The effect of miR-21 on the activation of astrocytes and retinal Müller cell and its molecular mechanism were studied by injecting miR-21 agomir and miR-21 antagomir into the vitreous cavity of rats with optic nerve injury.

3. miR-21 agomir and miR-21 antagomir were injected into the vitreous cavity of rats with optic nerve injury respectively to detect the axon regeneration of optic nerve, RGC survival and recovery of visual function.

Results:

1. Overexpression of miR-21 increased the number and bifurcation length of astrocytes and GFAP expression. The expression of Timp3 decreased, and the expression of p-EGFR, p-PI3K, p-AKT, p-mTOR and GFAP increased. Inhibition of miR-21 produced the opposite results.

2. miR-21 had no significant effect on the resting state of optic nerve astrocytes. After optic nerve injury, inhibition of miR-21 led to the hypertrophy and decrease of astrocytes number, increased expression of Timp-3 and decreased expression of p-EGFR, p-PI3K, p-AKT, p-mTOR, CSPG and GFAP. Overexpression of miR-21 produced the opposite results.

3. EGFRsiRNA, LY294002 and Rapamycin "reversed" miR-21 agomir-induced elevation of GFAP.

4. After optic nerve injury, inhibition of miR-21 led to the increase of gap-43 expression and the improvement of visual evoked potential function.

5. In the early stage of optic nerve injury (14 days), inhibition of miR-21 promotes "conservative gliosis" of Müller cell. At the later stage of injury (35 days), inhibition of miR-21 inhibited the "excessive gliosis" of Müller cell.

6. Inhibition of miR-21 increased the number of RGC and the thickness of retinal nerve fiber layer.

Conclusions:

1. Inhibition of miR-21 promotes excessive activation of astrocytes by

regulating the EGFR/PI3K/AKT/mTOR signaling pathway.

2. After optic nerve injury, inhibition of miR-21 can help reduce excessive activation of astrocytes and glial scar formation by regulating the EGFR/PI3K/AKT/mTOR pathway, thus promoting axonal regeneration of optic nerve and the repair of visual evoked potential.

3. In the early stage of optic nerve injury (14 days), inhibition of miR-21 promotes the activation of favorable Müller cell, while in the later stage of injury (35 days), inhibition of miR-21 inhibits the activation of unfavorable Müller cell.

4. After optic nerve injury, inhibition of miR-21 contributes to the increase in the number of RGC and the thickness of retinal nerve fiber layer, and improves visual function.

Keywords: Optic nerve injury, astrocyte, miR-21, EGFR/PI3K/AKT signaling pathway, axon regeneration