Title: **EGFR+ neural stem cell-derived exosomal microRNA-210-5p promotes axon regeneration after spinal cord injury via HDAC6/PI3K-AKT-mTOR axis**

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Abstract

Objective: To investigate the effect and molecular mechanisms of EFGR+ neural stem cells-derived exosomes (EGFR+ NSC-Exos) on spinal cord injury (SCI) in mice.

Methods：EGFG+ NSCs subtype were isolated by flow cytometry. Exosomes were extracted and purified by ultracentrifugation and characterized by electron microscopy, particle size analysis and western blot. We further used high-throughput sequencing to detect microRNAs profiles of NSC-Exos and EGFR+ NSC-Exos and validated significantly expressed microRNA by qRT-PCR and FISH. BMS scores, electrophysiology, immunofluorescence and HE staining were applied to evaluate the efficacy of exosomes in the treatment of spinal cord injury. Dual luciferase report was applied to explore downstream target of microRNA. Western blot and immunofluorescence staining were used to detect related signalling pathway proteins.

Results: EGFR+NSCs showed stronger ability of neural differentiation than primary-cultured neural stem cell. Functional assessment suggested that EGFR+ NSC-Exos had a superior therapeutic effect than primary NSC-Exos. Meanwhile, EGFR+ NSC-Exos could significantly promote autophagy and facilitate axon growth in vivo and in vitro. The result of high-throughput sequencing showed that microRNA-210-5p was significantly expressed in EGFR+ NSC-Exos. In neurons, microRNA-210-5p could bind to HDAC6 and negatively regulate its expression. Downregulation of HDAC6 further activated PI3K-AKT-mTOR signal pathway, promoted autophagy and improved microtubule stability of neurons. Use of antagomir-210-5p (inhibitor of microRNA-210-5p), HDAC6 plasmid (overexpression of HDAC6) or PKI-402 (inhibitor of PI3K and mTOR) abolished the therapeutic effect of EGFR+ NSC-Exos.

Conclusion: EGFR+NSC-Exos could transport microRNA-210-5p to neurons and downregulate expression of HDAC6. Down-regulation of HDAC6 activated PI3K-AKT-mTOR signal pathway, enhanced autophagy and microtubule stability of neurons after SCI. Our study provides a potential strategy of treatment of SCI.