The oxygen-sensing pathway in neural cells controls intrinsic long-term neuroregeneration after ischemic stroke

Pinar Kilic, Hugo H. Marti, Reiner Kunze

Institute of Physiology and Pathophysiology, Department of Cardiovascular Physiology, Heidelberg University, Heidelberg, Germany

Question

Cerebral hypoxia, a hallmark of ischemic stroke, initiates adaptive endogenous processes that mitigate acute neuronal cell death and promote long-term neuroregeneration. The latter involves upregulation of genes that drive angiogenesis, neurogenesis, and neuroplasticity through the activation of hypoxia-inducible factors (HIFs), whose activity is regulated by prolyl-4-hydroxylase domain (PHD) proteins in an oxygen-dependent manner. The present project aims to investigate whether the PHD-HIF axis regulates long-term regenerative processes in the stroke-damaged brain, and, thus, might serve as a potential molecular target for a pharmacological neurorestorative stroke therapy.

Methods

The tamoxifen-inducible Cre-loxP system was used to generate transgenic mice deficient for (i) PHD2 (*inPhd2*$^{\Delta/\Delta}$) and (ii) HIF-1α/HIF-2α (*inHif1a/Hif2a*$^{\Delta\Delta/\Delta\Delta}$) in neurons. In a pharmacological approach, C57BL/6 mice were treated with Roxadustat, a low-molecular-weight and blood-brain barrier permeable PHD inhibitor. A low and high dosage of Roxadustat or vehicle solution were systemically applied to mice three times a week from day 4 to 15 post-stroke. Mice were subjected to either transient middle cerebral artery occlusion or sham surgery followed by 42-56 days of reperfusion. The sensorimotor function was determined by evaluation of a modified Neurological Severity Score, Rotarod motor performance test, adhesive removal test, and pole test. Cresyl violet staining was applied to quantify brain tissue atrophy.

Results

In comparison to *Phd2*$^{f/f}$ littermates, *inPhd2*$^{\Delta/\Delta}$ mice showed markedly reduced sensorimotor impairment from subacute to chronic stages after stroke. In contrast, *inHif1a/Hif2a*$^{\Delta\Delta/\Delta\Delta}$ mice exhibited more severe sensorimotor impairment as compared to *Hif1a/Hif2a*$^{ff/ff}$ littermates. Delayed pharmacological activation of the HIF pathway in the brain by both low and high dosed Roxadustat substantially improved the sensorimotor performance during subacute and chronic stages after stroke compared to vehicle-treated animals. Interestingly, the extent of brain tissue atrophy due to ischemic stroke was not affected by either activation or inactivation of the HIF

pathway, suggesting that HIF-dependent processes improve functional recovery primarily through triggering compensatory neural rewiring in stroke-denervated areas.

Conclusion

Based on these findings, we hypothesize that the HIF pathway in brain-resident cells promotes adaptive neuroplasticity after stroke. In addition, our results may provide the basis for a new therapeutic approach to promote the long-term regeneration of sensorimotor functions upon ischemic stroke with a drug that is already approved for the treatment of chronic diseases in humans. Further experiments including ribosome tagging, anterograde axonal tracing, and histological analysis will be conducted to investigate the plasticity-related gene network and axonal as well as dendritic plasticity.