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TITLE: RNA-BINDING PROTEIN RBM3 CAN PREVENT MODERATE HYPOXIA-INDUCED CELL APOPTOSIS AND CELL CYCLE ARREST IN NEURAL STEM CELLS

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CONTENT:

Neonatal hypoxia-ischemia is a major cause of long-term neurological impairment. Neural stem cells (NSCs) reside physiologically in hypoxic niches and play important roles in neuro-regeneration after hypoxia-ischemic injury. While mild hypoxia appears to have proliferation-promoting effects on NSCs, moderate to severe hypoxia seems to show reverse effects. The cold-inducible RNA-binding motif protein 3 (RBM3) has multiple cellular functions including the regulation of apoptosis, cell proliferation, and cell cycle and promoting protein translation in NSCs. However, it is controversial whether and how it is participating in the oxygen stress response.

P10 SD rats were subjected to right common carotid artery ligation and then exposed to 8% O2 for 1 h. Left and right hemispheres were isolated for Western blot. Hypoxia-induced changes in RBM3 gene expression was assessed in mouse NSC line C17.2 by qPCR and Western blot. Cells were exposed for 24 h to different doses of hypoxia (1% to 18% O2) and either stained with Annexin V/propidium iodide (PI) without fixation or labeled with viability staining reagent and PI after fixation and then analyzed by fluorescence-activated cell sorting (FACS). To examine the role of RBM3 in hypoxia-induced apoptosis and cell cycle arrest, C17.2 cells were transfected with empty or RBM3-overexpressing vector and then exposed to hypoxia for 24 h. Statistical analysis: unpaired two-sample t-test (P < 0.05).

Hypoxia had a dose-dependent suppressive effect on proliferation of C17.2 NSCs by increasing the number of cells in G1 phase when measured after 24h hours. This resulted in G1 cell cycle arrest under moderate (2.5% O2) and severe hypoxia (1% O2) and to reduced cell survival by increased apoptosis and necrosis. In vivo, RBM3 expression decreased after HI compared to sham group. In ipsilateral site of stroke, RBM3 level was higher compared to contralateral site. In vitro, RBM3 gene expression decreased in NSCs by about 50% under very mild hypoxia and showed only a small further decrease under moderate to severe hypoxia. Exogenous (vector) RBM3 overexpression significantly blocked cell cycle by decreasing the cell number in G1 phase and increased the cell number in S phase compared to controls. Also, RBM3 slightly increased cell viability but had no significant effect on apoptosis.

The expression of the cold-inducible protein RBM3 is sensitively regulated in NSCs in response to hypoxia. Exogenous upregulation of RBM3 can prevent NSCs from getting arrested in G1 phase under moderate hypoxia levels. These findings suggest that RBM3 is involved in NSC proliferation in a hypoxic environment.

COI: None declared
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TITLE: MESENCHYMAL STEM CELL-DERIVED VESICLES FOR TREATMENT OF NEONATAL HYPOXIC-ISCHEMIC BRAIN INJURY
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CONTENT:
Neonatal encephalopathy caused by hypoxia-ischemia (HI) is a major cause of death and disability in newborns. Stem cell-based regenerative therapies seem promising to prevent long-term neurological deficits. Our previous work in neonatal HI revealed unexpected risks of mesenchymal stem cell (MSC) therapy due to interaction with the brains’ microenvironment. An alternative to cell therapy may be the use of MSC-derived extracellular vesicles (MSC-EV). According to our recent studies in models of adult stroke and inflammation-induced perinatal brain injury demonstrating therapeutic effects of MSC-EV, we hypothesized that MSC-EV promote neuroregeneration in neonatal HI-induced brain injury.

Nine day old C57BL/6 mice were exposed to HI through ligation of the right common carotid artery followed by one hour hypoxia (10% oxygen). MSC-EV (1x10^5 cell equivalents) or vehicle control (0.9% saline) were injected intraperitoneally immediately after HI. Seven days after HI, brain injury was assessed by regional neuropathological scoring and atrophy measurements in cresyl violet stained tissue sections. Immunohistochemistry for NeuN, Olig2 and CD31 was applied to assess effects on neurons, oligodendrocyte and vessel densities, respectively. Cell proliferation was analysed in tissue sections stained for the proliferation marker Ki-67. Analysis was performed via confocal imaging (Nikon A1plus, Eclipse Ti) followed by automated cell counting with the respective NS1 analysis software.

While total neuropathological scores were not significantly changed, regional analysis of HI-induced brain injury revealed a significant protection from striatal tissue loss in MSC-EV-treated animals seven days after HI. Furthermore, cell-specific analyses via immunohistochemistry demonstrated that reduced tissue atrophy was accompanied by an increased neuronal, oligodendrocyte and endothelial density in the striatum. Interestingly, larger cell densities in MSC-EV-treated mice were associated with a significantly enhanced amount of proliferating cells in the neurogenic sub-ventricular zone close to the striatum.

These data indicate that MSC-EV promote neuroregeneration by inducing cell proliferation in neurogenic niches of the injured neonatal brain resulting in increased cell densities of a variety of CNS cells thereby preventing secondary HI-induced brain tissue loss. Considering potential unforeseen risks of stem cell therapy, these data suggest that MSC-EV may be a promising alternative to cell therapy for neonatal brain injury.

COI: None declared