

Insights into remote ischemic conditioning miRNA effects on brain endothelial cells in an oxygen-glucose deprivation stroke model

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Background

- Acute ischemic stroke (AIS): Treatment window of 4.5-6 hours.
- Extracellular vesicles (EVs) from Remote ischemic conditioning (**RIC**) and blood flow restricted resistance exercise (BFRRE) protect human brain microvascular endothelial cells (HBMECs) from ischemiareperfusion (I/R) injury
- The microRNA (miRNA) from EVs alter gene expression at targets cells and may be the driver of protection
- Model: HBMECs exposed to oxygen-glucose deprivation (OGD) to model ischemic stroke *in vitro*





Gene profile change due to RIC-miRNA transfection and OGD

Both transfection of HBMECs with RIC-miRNAs OGD resulted in changes in mRNA expression

larger differences than oxygen tension in terms of variance.



The common and unique differentially expressed genes (DEGs) in all of the analyses.

OGD-RIC-miRNAs targets neuronal degradation and cell cycle processes



RIC-miRNAs effect in I/R injury *in vitro*



Human Brain Microvascular Endothelial Cells (HBMECs) transfected with RIC-miRNAs showed similar viability to untransfected naïve HBMECs (p-value = 0.23) after OGD (3h ischemia + 4h reperfusion), whereas the viability of NC-miRNA transfected HBMECs was significantly lower when compared with untransfected naïve HBMECs (p-value = 0.02). No significant difference was found between the RIC-miRNA and NC-miRNA groups. N = 3 in 3 consecutive trials.





NC-miRNAs OGD vs Non-OGD

associated with cell cycle activation and DNA replication.

No transfection: Untransfected HBMECs in complete growth media with serum. All other cells are grown in reduced medium without serum. Data is normalized to untransfected HBMECs within each experiment. Data shown as mean ±SD.



Upregulated CDC25A G₀ phase (restina) Cyclin E1 Cyclin E2 'G1 checkpoint G₁/S checkpoint MCM2 MCM4 MCM6 MCM10 G₂/M checkpoint CDC6

OGD-RIC-miRNA activates cell cycle Lower levels of Na⁺ and the presence of IL-12 To conserve energy during hypoxia, cell cycle leads to swelling of neurons and accelerated progression is inhibited. OGD-RIC-miRNAs upregulate drivers of cell RIC-miRNAs may act on Na⁺ transport and inhibit cycle activation, leading to more cell divisions IL-12 production during the acute stroke phase. and proliferation.

Conclusion

and regulation of pro-inflammatory IL-12.

Funding and references

neuronal degradation.

- RIC-miRNAs introduce many mRNA changes in HBMECs both at OGD and normoxic conditions \bullet
- RIC-miRNAs target ion transport and inhibit IL-12 production, while the derivative effects of the RIC-miRNAs at OGD \bullet conditions lead to increased cell cycle activation and DNA replication
- This point towards an early response to the RIC-miRNAs from the downregulated genes, while the upregulated \bullet genes are involved in beneficial pathways with a longer horizon.

adjusted p-value < 0.05. Total =14516 genes.

- RIC-miRNAs help HBMECs return to normal physiological conditions after OGD
- These pathways may be targets for new therapeutic avenues

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