PERSPECTIVE

Exploring and exploiting unique properties of the hippocampal dentate gyrus for post-stroke therapy: astrocytes link ischemic resistance with neurogenic potential

Acute cerebral ischemia can occur secondary to embolism, cardiac arrest, hemorrhage, traumatic brain injury, edema, vascular compression, or any physiologic condition resulting in low cardiac output state. Survivors of cerebral ischemic events frequently suffer from profound disability, accounting for > $70 billion in 2010 for treatment of embolic stroke in the US alone. More strikingly, the cost to treat stroke in the US is anticipated to soar to > $180 billion by 2030, according to estimates from the American Heart Association (Ovbiagele et al., 2013). Despite hundreds of promising pre-clinical studies in animal models, effective clinical treatments for the most common forms of cerebral ischemia remain limited: standard treatment for embolic stroke is restricted to thrombolytic therapy or clot retrieval during a narrow therapeutic window, while post-resuscitation treatment following cardiac arrest remains the (controversial) application of mild hypothermia. The massive failure to translate experimental findings to the development of any successful clinical intervention suggests that redirecting investigational resources to developing new therapies to improve long-term recovery in survivors of stroke may be a logical and necessary next step.

The hippocampal dentate gyrus (DG) and the subventricular zone of the anterolateral ventricle are the two primary regions in the adult mammalian brain that maintain active neurogenesis, and cerebral ischemia has been shown to stimulate adult neurogenic activity in both of these regions (Li et al., 2010). Appropriately, targeting the neurogenic niche has been a recent focus for the development of novel post-injury interventions to augment post-injury neurogenesis and improve long-term neurobehavioral outcome following cerebral ischemia (Marlier et al., 2015). Interestingly, neurons in the hippocampal DG subregion are also known to have a relatively higher ischemic resistance *versus* neurons in the adjacent cornu ammonis 1 (CA1) subregion. Delineating the mechanisms that determine observed differences in both neurogenic potential and sensitivity to ischemia between the CA1 and DG hippocampal subregions might provide new avenues in the development of post-injury therapies to improve long-term neurobehavioral outcome in survivors of all types of cerebral ischemia. Subregional analyses have demonstrated differences in neuronal gene expression between DG and CA1 (Lein et al., 2004), including differences in signal transduction pathways, transcription factors, calcium binding proteins, and genes maintaining a unique extracellular milieu. Most recently (Stary et al., 2016), we have observed that astrocytes, the most numerous type of cell in the brain and critical regulators of neuronal homeostasis, may also account for some of these observed subregional differences.

Astrocytes play many critical roles in supporting normal neuronal functioning, including maintaining ionic balance and modulating neurotransmission (Clarke and Barres, 2013), and astrocyte homeostasis is tightly coupled to neuronal cell fate

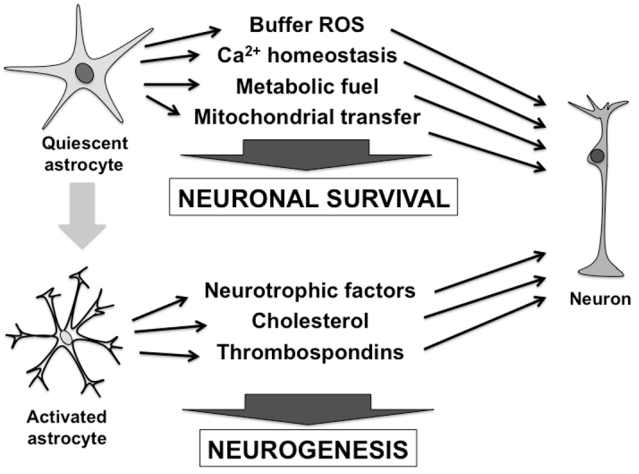

Figure 1 Astrocytes regulate neuronal survival and neurogenesis.
Astrocytes provide protection to neurons by a number of mitochondrial-associated mechanisms, including buffering excessive reactive oxygen species (ROS), maintaining calcium (Ca^{2+}) homeostasis, and providing metabolic substrate and ATP to neurons. Astrocytes may also regulate neuronal homeostasis and the neuronal bioenergetic response to injury by direct transfer of mitochondria from astrocytes to neurons. Following cerebral ischemia, astrocytes become activated and have the capacity to promote neurogenesis.

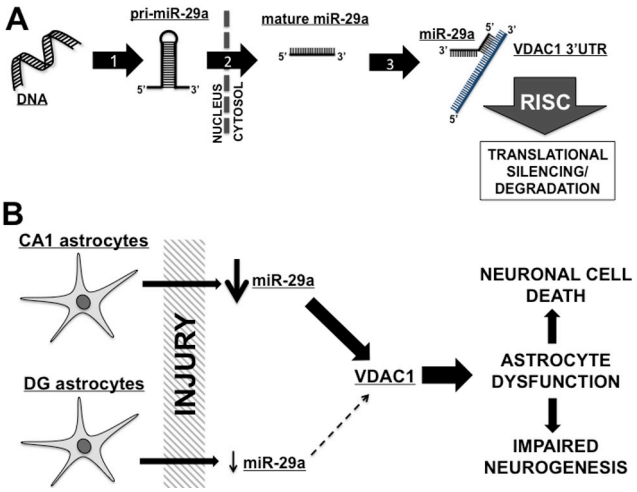

Figure 2 miR-29a targets voltage dependent anion channel-1 (VDAC1).
(A) miR-29a biogenesis begins in the nucleus with genomic transcription of pri-miR-29a (1). Drosha-mediated cleavage results in pre-miR-29a, which is then exported to the cytosol by Exportin-5 and processed to the final mature miR-29a form by Dicer (2). In the cytosol, miR-29a is then free to interact with the 3′ untranslated region of VDAC1 messenger RNA (3). miR-29a/VDAC1 complexes are then targeted by the RNA-induced silencing complex (RISC) for either VDAC1 mRNA degradation or translational silencing, depending on the degree of miR/mRNA binding complementarity. (B) A decrease in expression of miR-29a in response to injury in astrocytes results in an increase in VDAC1, inducing astrocyte dysfunction, which can contribute to neuronal cell death and ultimately impair long-term, post-injury neurogenesis. Astrocytes in the cornu ammonis-1 (CA1) hippocampal subregion have a greater decrease in miR-29a than astrocytes from the dentate gyrus (DG), in part explaining the select resistance of DG neurons to cerebral ischemia.

following ischemia-reperfusion (**Figure 1**) by buffering reactive oxygen species, maintaining Ca^{2+} homeostasis and ensuring adequate neuronal energy stores (Nedergaard and Dirnagl, 2005; Ouyang et al., 2014). Intriguingly, Hawakawa et al. (2016) recently demonstrated that astrocytes are also capable of direct transfer of functional mitochondria to neurons, and that suppressing this process worsens injury following cerebral ischemia. Astrocyte "activation" and glial scar formation post-injury have traditionally been considered detrimental to stroke recovery, but reactive astrocytes have more recently been demonstrated to have the capacity to promote neuroplasticity (**Figure 1**) *via* secretion of neurotrophic factors, cholesterol and thrombospondins (Liu and Chopp, 2015). Previous observations (Ouyang et al., 2007) suggest that astrocytes within the hippocampus CA1 are more sensitive to ischemic injury, and with a higher degree of mitochondrial dysfunction compared to DG astrocytes, and that disruption of mitochondrial homeostasis in local astrocytes following cerebral ischemia contributes to CA1 neuronal cell death (Xu et al., 2010; Ouyang et al., 2013). Together these observations demonstrate a potential role for mitochondria in neuronal-astrocyte communication, and position astrocytes as central for maintenance of neuronal metabolism and bioenergetics in response to cell stress and recovery from injury. Therefore, long-term post-injury gene therapy strategies aimed at maintaining astrocyte mitochondrial function may provide a novel approach to accelerate and magnify recovery from ischemic brain injury.

MicroRNAs (miRs) are an endogenous class of small, non-coding RNAs that modulate gene expression by binding to the 3' untranslated region (UTR) of target genes and destabilizing or inhibiting their translation (**Figure 2A**). microRNAs are an attractive therapeutic candidate for long-term modulation of neuronal and glial mitochondrial function as endogenous microRNA levels can be easily altered with chemically modified mimics and inhibitors. microRNA-29a (miR-29a) is enriched in astrocytes, and has been shown to be a critical regulator of neurogenesis during cortical development (Li et al., 2014). Targeted knockdown of miR-29a in the brain has been shown to induce hippocampal neuronal cell death in the adult brain (Roshan et al., 2014), mediated in part by targeting the voltage-dependent anion channel-1 (VDAC1), a critical regulator of mitochondrial function and cell survival. Most recently (Stary et al., 2016), we have demonstrated that astrocytes selectively cultured from the hippocampal DG subregion had a greater resistance to injury *versus* astrocytes cultured from the CA1, recapitulating *in vivo* observations of selective vulnerability of CA1 neurons to ischemia. This difference was due, at least in part, to a differential expression pattern in miR-29a/VDAC1 between subregions (**Figure 2B**), and was eliminated by blocking the interaction of miR-29a with the VDAC1 3'UTR. These findings suggest that microRNA biogenesis in astrocytes may provide a critical nexus between neuronal survival and post-injury neurogenic potential, and explain in part the unique properties of the DG subregion. Further exploring the differences between these two hippocampal subregions in baseline astrocyte physiology, and function in response to injury, may give rise to effective novel therapies for long-term recovery and repair of the injured brain following ischemic injury.

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References

Clarke LE, Barres BA (2013) Emerging roles of astrocytes in neural circuit development. Nat Rev Neurosci 14:311-321.

Hayakawa K, Esposito E, Wang X, Terasaki Y, Liu Y, Xing C, Ji X, Lo EH (2016) Transfer of mitochondria from astrocytes to neurons after stroke. Nature 535:551-555.

Lein ES, Zhao X, Gage FH (2004) Defining a molecular atlas of the hippocampus using DNA microarrays and high-throughput in situ hybridization. J Neurosci 24:3879-3889.

Li H, Mao S, Wang H, Zen K, Zhang C, Li L (2014) MicroRNA-29a modulates axon branching by targeting doublecortin in primary neurons. Protein Cell 5:160-169.

Li Y, Yu SP, Mohamad O, Genetta T, Wei L (2010) Sublethal transient global ischemia stimulates migration of neuroblasts and neurogenesis in mice. Transl Stroke Res 1:184-196.

Liu Z, Chopp M (2015) Astrocytes, therapeutic targets for neuroprotection and neurorestoration in ischemic stroke. Prog Neurobiol 144:103-120.

Marlier Q, Verteneuil S, Vandenbosch R, Malgrange B (2015) Mechanisms and functional significance of stroke-induced neurogenesis. Front Neurosci 9:458.

Nedergaard M, Dirnagl U (2005) Role of glial cells in cerebral ischemia. Glia 50:281-286.

Ouyang YB, Voloboueva LA, Xu LJ, Giffard RG (2007) Selective dysfunction of hippocampal CA1 astrocytes contributes to delayed neuronal damage after transient forebrain ischemia. J Neurosci 27:4253-4260.

Ouyang YB, Xu L, Liu S, Giffard RG (2014) Role of astrocytes in delayed neuronal death: GLT-1 and its novel regulation by microRNAs. Adv Neurobiol 11:171-188.

Ouyang YB, Xu L, Lu Y, Sun X, Yue S, Xiong XX, Giffard RG (2013) Astrocyte-enriched miR-29a targets PUMA and reduces neuronal vulnerability to forebrain ischemia. Glia 61:1784-1794.

Ovbiagele B, Goldstein LB, Higashida RT, Howard VJ, Johnston SC, Khavjou OA, Lackland DT, Lichtman JH, Mohl S, Sacco RL, Saver JL, Trogdon JG (2013) Forecasting the future of stroke in the United States: a policy statement from the American Heart Association and American Stroke Association. Stroke 44:2361-2375.

Roshan R, Shridhar S, Sarangdhar MA, Banik A, Chawla M, Garg M, Singh VP, Pillai B (2014). Brain-specific knockdown of miR-29 results in neuronal cell death and ataxia in mice. RNA 20:1287-1297.

Stary CM, Sun X, Ouyang Y, Li L, Giffard RG (2016) miR-29a differentially regulates cell survival in astrocytes from cornu ammonis-1 and dentate gyrus by targeting VDAC1. Mitochondrion 30:248-254.

Xu L, Emery JF, Ouyang YB, Voloboueva LA, Giffard RG (2010) Astrocyte targeted overexpression of Hsp72 or SOD2 reduces neuronal vulnerability to forebrain ischemia. Glia 58:1042-1049.