



## Commentary

# The silencing of circular RNA in neural stem cells – A gateway to new therapeutic strategies in cerebral ischemia?

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## ARTICLE INFO

## Article History:

Received 21 February 2020

Accepted 21 February 2020

Available online xxx

In this article of *EBioMedicine*, Guangtian Wang et al. describe the effects of the circular RNA homeodomain-interacting protein kinase 2 (circHIPK2) on neural stem cells (NSC) and on neurons generated from NSC, both *in vitro* and in a mouse model of focal cerebral ischemia [1].

After their initial description in the 1970s, our understanding of circular RNAs as endogenous noncoding RNAs has vastly grown, also thanks to the advent of next-generation sequencing in the early 2000s [2]. The 3' and 5' ends of this particular type of RNA are covalently linked and formed by alternative splicing, a phenomenon called 'backsplicing' [3]. In that process, non-coding intronic sequences are spliced and released as molecules with regulatory functions. Due to the lack of free ends, circular RNAs are more stable than linear RNAs and can consequently circumvent degradation by enzymes [2]. They were shown to be not only a transcriptional product in numerous human and mouse genes, but to also constitute the dominant RNA isoform [3]. CircRNAs appear to serve as microRNA sponges and reduce the activity of corresponding miRNAs by directly binding them. This results in the regulation of target genes. Localised in the cytoplasm according to a cell type specific expression, recent studies have identified circRNAs as potential prognostic and diagnostic biomarkers as well as therapeutic targets in various neurological diseases including neurodegenerative disorders and neoplasia [4]. Interestingly, the expression profile of circRNAs is also altered following cerebral ischemia, suggesting not only implications to post-stroke pathophysiology, but potentially also constituting a novel therapeutic target [5].

Located mainly in specialized niches in the adult mammalian brain, NSC have the ability to self-renew and differentiate into functional neurons, astrocytes and oligodendrocytes throughout the lifespan. In regenerative medicine, strategies are being developed to

either (i) transplant stem cells aiming at cell replacement, or to (ii) harness the regenerative capacity of the brain by mobilizing the endogenous NSC niche [6]. Wang et al. investigated the effect of HIPK2 on NSC differentiation in the context of cerebral ischemia. HIPK2 is a "caretaker" gene with major presence in brain cells and is responsible for a wide spectrum of biological functions such as cell proliferation, invasion, hypoxia, apoptosis, and DNA damage response [7]. Wang et al. demonstrate that circHIPK2 reduces neuronal fate in differentiating NSC, while silencing circHIPK2 induces neurogenesis *in vitro*, with no apparent effect on the development of astrocytes [1]. NSC-derived neurons, especially their processes and dendritic spines, are protected from ischemic stress by si-circHIPK2 in an *in vitro* model of cerebral ischemia induced by oxygen- and glucose-deprivation (OGD). Likewise, in an *in vivo* mouse model of cerebral ischemia by transient middle cerebral artery occlusion (tMCAO), silencing of the circHIPK2 leads to increased neuronal plasticity in the ischemic brain, resulting in neuroprotection and reduction of functional deficits. These effects are mediated through down-regulation of spermine oxidase (Smox), a mediator involved in ischemic brain damage [1]. The latter result is in line with a recent study describing Smox downregulation to reduce brain infarct volume, downregulate neuroinflammatory processes, and ameliorate neurological deficits in stroke rats [8].

Another recent study achieved beneficial effects on infarct volume and functional recovery from stroke by silencing of a circRNA (i.e., circTLK1) [9]. In this study, silencing of circTLK1 was achieved by direct intracerebroventricular injection of circTLK1 shRNA lentivirus in a mouse model of tMCAO, notably one week before induction of cerebral ischemia [9]. In this study in *EBioMedicine*, Wang et al. for the first time combine a cell-therapy approach (the transplantation of NSC) with the silencing of circRNA (within the transplanted cells), and apply this treatment one week after induction of cerebral ischemia, thus in the subacute phase of stroke. Intriguingly, this phase is characterized by a cascade of sterile neuroinflammatory processes with substantial impact on NSC-mediated regeneration [10]. Thus,

DOI of original article: <http://dx.doi.org/10.1016/j.ebiom.2020.102660>.

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<https://doi.org/10.1016/j.ebiom.2020.102705>

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great potential lies in future studies on the immunomodulatory effects of si-circHIPK2-NSC and – vice versa – on the effects of post-ischemic neuroinflammation on survival and differentiation of the transplanted cells. Potentially, circRNAs could be an attractive way to modulate NSC function in order to support brain regeneration and facilitate functional recovery from cerebral ischemia, perhaps even in the subacute and chronic stages of stroke.

#### Authors' contributions

DNO drafted and MAR finalized the manuscript.

#### Declaration of Competing Interest

The authors declare that they have nothing to disclose.

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