Journal Club

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Delayed Treatment with Histone Deacetylase Inhibitors Promotes Stroke Recovery

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Review of Lin et al.

Stroke is a leading cause of death and disability worldwide (Benjamin et al., 2017). Significant advances in hyper-acute care (<24 h after stroke; Bernhardt et al., 2017), including the administration of recombinant tissue plasminogen activator and thrombolytic and endovascular surgery (Prabhakaran et al., 2015), have resulted in declining rates of mortality. Improved survival, however, has brought new challenges in treating long-term disability. Unfortunately, the search for effective therapeutic options that can be used at later time points (>24 h after stroke onset) has thus far resulted in high-profile and costly failures (e.g., NMDA receptor antagonists), highlighting the urgent need for novel options to improve the devastating functional deficits present after stroke.

Recent work has highlighted the possible contribution of post-translational modifications of histones via histone deacetylases (HDACs) to disease and injury states, including that resulting from stroke (Chuang et al., 2009). HDACs remove the acetyl group on histones and work synchronously with other histone-modifying enzymes to regulate gene transcription, ensuring that cells respond appropriately to their needs and changes in environmental conditions (Robert et al., 2011). However, HDACs can be rendered dysfunctional under neuropathological conditions and have been implicated in Parkinson's disease, Alzheimer's disease, and stroke (Chuang et al., 2009).

In stroke, the role of HDACs has been examined predominantly in the context of neuroprotection, at the hyper-acute phase. For example, pan-HDAC inhibitors such as valproic acid, sodium butyrate, or trichostatin A, can decrease stroke-induced infarct volume and reduce inflammation when administered within 3 h after stroke induction (Kim et al., 2007). Another study examining the role of HDACs after oxygen and glucose deprivation in cultured mouse optic nerves found that the administration of the class I HDAC inhibitor suberoylanilide hydroxamic acid before ischemia could confer neuroprotection by preserving white matter integrity (Baltan et al., 2011). While HDAC inhibitors administered at hyper-acute time points are an important therapeutic option, reports of long-lasting histone hypoacetylation due to HDAC and histone acetyltransferase dysfunction after stroke (Park and Sohrabji, 2016) indicate the possible effectiveness of HDAC inhibitors at acute (1–7 d) time points as well.

In a recent report in *The Journal of Neuroscience*, Lin et al. (2017) examined the role of HDACs in focal ischemia by monitoring and modulating HDAC2 function at the acute stage of stroke recovery. Lin et al. (2017) produced cortical strokes in mice and monitored sensorimotor performance on two behavioral tasks: a grid walk task, which evaluates limb placement and coordination, and the cylinder task, which evaluates spontaneous forelimb use. As expected, motor deficits were clearly present 1 d after stroke induction, but they improved at 2–4 d after stroke. Unexpectedly, Lin et al. (2017) reported that behavioral outcome worsened from 5 to 7 d after stroke, naming this period "the secondary functional loss" phase. Previous work has described a secondary loss phase, where progressive tissue damage and loss of neurons and glia are observed after the immediate injury (Dirnagl et al., 1999; Zhang et al., 2012). While it stands to reason that this secondary loss phase might be accompanied by behavioral deficits, several other groups have not identified a secondary functional loss phase in a variety of stroke and injury models (van Meer et al., 2010; Liu et al., 2014a,b; Hiu et al., 2016). The reasons why Lin et al. (2017) were able to identifying this secondary functional loss phase are unclear; however, daily behavioral evaluation com-

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mencing immediately after stroke onset may have exacerbated stroke-induced motor deficits and/or evoked a secondary event such as hemorrhagic transformation, manifesting into a secondary functional loss. Consistent with this idea, a large-scale multicenter randomized controlled trial (AVERT Trial Collaboration Group, 2015) recently revealed that early rehabilitative intervention may negatively impact outcome in stroke patients. Further experiments reducing the amount of behavioral assessment following stroke are needed to determine whether early intervention is contributing to the appearance of secondary functional loss. Regardless, analysis of the regions neighboring the infarct, termed the peri-infarct area, after stroke revealed that HDAC2 activity was specifically increased during this secondary functional loss phase.

To determine whether the upregulation of HDAC2 contributes to secondary functional loss after stroke, Lin et al. (2017) implanted a microcannula into the peri-infarct cortex and introduced inhibitors targeting different classes of HDACs. Infusion of the selective class I HDAC inhibitor MGCD0103 persistently improved stroke-induced motor deficits, but this effect was observed only when the inhibitor was introduced during the secondary functional loss phase (i.e., 5–7 d after stroke). Notably, Lin et al. (2017) also improved motor deficits using a Food and Drug Administration-approved drug normally used to target HDAC1 and HDAC2 for cancer treatment.

Next, Lin et al. (2017) determined whether HDAC2 expression alone could alter functional outcome after stroke. First, they infused adeno-associated viruses (AAVs) into the peri-infarct cortex to abolish or overexpress HDAC2 and demonstrated that stroke-induced motor deficits were correlated with HDAC2 expression in peri-infarct cells. Second, they deleted HDAC2 from forebrain cells and showed that these transgenic mice demonstrated an improved functional outcome similar to that of animals treated with the HDAC2 inhibitor, as well as a worsened functional outcome when viral overexpression of HDAC2 was induced in the peri-infarct cortex.

To better understand how HDAC2 inhibitors ameliorate secondary functional loss after stroke, Lin et al. (2017) compared gene expression from the sham-injured cortex and the stroke-injured cortex treated with either vehicle or the class I HDAC inhibitor MGCD0103. One gene that was upregulated after stroke and downregulated after HDAC inhibitor treatment was bestrophin 1 (BEST1), an anion channel capable of transporting GABA, and contributes to the tonic GABAergic tone in the cortex (Lee et al., 2010). Several reports have recognized the critical role for GABAergic signaling in the cortex following focal stroke (Clarkson et al., 2010; Hiu et al., 2016). In particular, Clarkson et al. (2010) demonstrated an increased tonic GABA-mediated inhibition present in layer II cortical neurons after stroke that worsened functional recovery. Lin et al. (2017) showed that the stroke-induced increase in tonic GABA inhibition was reversed in HDAC inhibitor-treated mice.

Overall, Lin et al. (2017) put forth the idea that the stroke-induced upregulation of HDAC2 within the peri-infarct cortex strengthens GABAergic tone via BEST1, resulting in a secondary functional loss phase. One important question remains unanswered: what type of cell contributes to this effect? HDAC2 expression is present in neurons, astrocytes, and oligodendrocytes (for review, see Gräff and Tsai, 2013), and the AAV plasmid used by Lin et al. (2017) has a notably high transduction efficiency for cortical glia (Aschauer et al., 2013). Thus, it is possible that reactive astrocytes, which are present in high numbers within peri-infarct regions after stroke (Li et al., 2014), may act as key contributors to the secondary functional loss. Interestingly, a recent report (Maiarù et al., 2016) demonstrated that HDAC2 is increased in dorsal horn astrocytes, but not neurons, after spared nerve injury. In addition, the BEST1 channel is strongly expressed in astrocytes and is redistributed within the cell under pathological conditions (Park et al., 2009; Oh and Lee, 2017). This presents the hypothesis that ischemia may cause BEST1-containing reactive astrocytes to upregulate HDAC2 and worsen motor deficits after stroke. Further experiments identifying the role of HDACs in reactive astrocytes may provide some insight into this hypothesis.

All together, Lin et al. (2017) provide a strong argument for HDAC2 inhibitors as a candidate to improve stroke recovery. Despite some uncertainty regarding the cell types involved in HDAC2 expression, Lin et al. (2017) clearly demonstrate that a delayed treatment of HDAC inhibitors can have profound and lasting effects on behavioral deficits after stroke. Thus, this article introduces a possible therapy that may improve the quality of life for survivors of stroke.

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