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Histone deacetylase expression in white matter oligodendrocytes after stroke

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ABSTRACT

Histone deacetylases (HDACs) constitute a super-family of enzymes grouped into four major classes (Class I–IV) that deacetylate histone tails leading to chromatin condensation and gene repression. Whether stroke-induced oligodendrogenesis is related to the expression of individual HDACs in the oligodendrocyte lineage has not been investigated. We found that 2 days after stroke, oligodendrocyte progenitor cells (OPCs) and mature oligodendrocytes (OLGs) were substantially reduced in the peri-infarct corpus callosum, whereas at 7 days after stroke, a robust increase in OPCs and OLGs was observed. Ischemic brains isolated from rats sacrificed 7 days after stroke were used to test levels of individual members of Class I (1 and 2) and Class II (4 and 5) HDACs in white matter oligodendrocytes during stroke-induced oligodendrogenesis. Double immunohistochemistry analysis revealed that stroke substantially increased the number of NG2+ OPCs with nuclear HDAC1 and HDAC2 immunoreactivity and cytoplasmic HDAC4 which were associated with augmentation of proliferating OPCs, as determined by BrdU and Ki67 double reactive cells after stroke. A decrease in HDAC1 and an increase in HDAC2 immunoreactivity were detected in mature adenomatous polyposis coli (APC) positive OLGs, which paralleled an increase in newly generated BrdU positive OLGs in the peri-infarct corpus callosum. Concurrently, stroke substantially decreased the acetylation levels of histones H3 and H4 in both OPCs and OLGs. Taken together, these findings demonstrate that stroke induces distinct profiles of Class I and Class II HDACs in white matter OPCs and OLGs, suggesting that the individual members of Class I and II HDACs play divergent roles in the regulation of OPC proliferation and differentiation during brain repair after stroke.

1. Introduction

White matter consists mostly of glial cells and myelinated axons. It comprises about half of the brain volume in humans, and nearly all cases of ischemic stroke involve white matter (Goldberg and Ransom, 2003; Liu et al., 2012). Mature oligodendrocytes (OLGs), the glial cells responsible for CNS myelin formation, are highly vulnerable to ischemic injury mediated by oxidative stress, excitatory amino acids, trophic factor deprivation and apoptosis (Dewar et al., 2003; Pantoni et al., 1996). OLG injury results in demyelination with subsequent impairment of axonal conduction (Franklin and Ffrench-Constant, 2008; McTigue and Tripathi, 2008).

Myelin repair involves the generation of new mature OLGs, since surviving OLGs after injury are incapable of playing a significant role in remyelination (Franklin and Ffrench-Constant, 2008; Keirstead and Blakemore, 1997; McTigue and Tripathi, 2008). New OLGs are derived from non myelinating oligodendrocyte progenitor cells (OPCs) located throughout the grey and white matter of the adult brain (Franklin and Ffrench-Constant, 2008; McTigue and Tripathi, 2008). New OLGs are generated in the peri-infarct area in animal models of stroke (Gregersen et al., 2001; Tanaka et al., 2003; Zhang et al., 2010, 2011), however, the molecular

Abbreviations: Ac-HH, acetylated histone H; APC, adenomatous polyposis coli; BrdU, bromodeoxyuridine; DAPI, 4,6'-diamidino-2-phenylindole; HDAC, histone deacetylase; MBP, myelin basic protein; MCAO, middle cerebral artery occlusion; NG2, chondroitin sulfate proteoglycan; OLGs, mature oligodendrocytes; OPCs, oligodendrocyte progenitor cells; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

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mechanisms underlying stroke-induced oligodendrogenesis have not been extensively investigated (Zhang et al., 2013).

Histone deacetylases (HDACs) comprise a super-family of enzymes grouped into four major classes (Class I–IV) that deacetylate specific lysine residues in histone tails leading to chromatin condensation and gene repression (Jenuwein and Allis, 2001; Kouzarides, 2007). Numerous studies suggest that the functions and expression profiles of HDAC isoforms in oligodendrocytes dynamically respond to the developmental stage, age and health of the cells. Developmental animal studies revealed that all Class I and Class II HDAC isoforms exist in the corpus callosum at different developmental time points up to 24 days postnatally (Shen et al., 2005). The enzymatic activity of HDACs on nucleosomal histones was found to be essential for embryonic human and rodent OPCs differentiation (Conway et al., 2012; Marin-Husstege et al., 2002; Shen et al., 2005) and systemic administration of valproic acid (VPA), a non-selective HDAC inhibitor, to rat pups prevented the differentiation of developing OPCs and resulted in significant hypomyelination (Shen et al., 2005). However, the deleterious effect of treatment with non-selective HDAC inhibitors on oligodendrocytes in vivo is temporally restricted and takes place up to the first 10 postnatal days (Shen et al., 2005). Other evidence also suggests that the aging process affects histone acetylation in white matter oligodendrocytes. For example, Shen et al., (2008a) found decreased HDAC enzymatic activity in protein extracts of the corpus callosum of aged mice (8 months old) compared to young mice (8 weeks old) along with a generalized age-dependent decrease of Class I and Class II HDACs expression in OLGs.

Preclinical studies in animal models of stroke showed that inhibition of HDACs provides neuroprotection (Kim et al., 2007; Ren et al., 2004), stimulates neurogenesis and increases white matter repair (Kim et al., 2009; Liu et al., 2012). Treatment of adult stroke rats with VPA increases mature OLG survival by reduction of apoptotic OLG death and augmentation of newly generated oligodendrocytes (Liu et al., 2012). Similarly, two in vitro studies showed that treatment with HDAC inhibitors preserves mature oligodendrocytes and promotes functional recovery in optic nerves from young and aged mice after oxygen and glucose deprivation (OGD) (Baltan, 2012; Baltan et al., 2011). Most of these investigations on HDAC function have been based on non-selective HDAC inhibitors and their effect on white matter after ischemia. The HDAC family has only recently started to be addressed individually, and emerging data suggest that individual HDACs have distinctive biological functions in oligodendrocytes. Single knockouts of Class I HDAC1 or HDAC2 under the control of Olig1 promoter in mice did not yield obvious developmental defects, however, double genetic ablation of HDAC1 and HDAC2 blocked oligodendrocyte differentiation by activation of the Wnt signaling pathway (Ye et al., 2009). In another study, Wei et al. showed that transcription factor Nkx2.2 recruits an HDAC1 complex to repress the myelin basic protein (MBP) promoter in immature oligodendrocytes (Wei et al., 2005). Unlike Class I HDACs, the roles of Class II HDACs in oligodendrocyte development are still not known (Yao and Yang, 2011). Other reports investigated roles of individual Class III and IV HDACs in oligodendrocyte differentiation; Class III HDAC Sirt2 promotes the differentiation of CG4 oligodendrocytes (Ji et al., 2011), and knockdown of the Class IV HDAC (HDAC11) reduces myelin gene expression and arrested oligodendrocyte maturation in the same cell line (Liu et al., 2009).

In the present study, using a rat model of focal cerebral ischemia, we examined the spatial profile of individual Class I and Class II HDACs in OPCs and mature OLGs in the peri-infarct white matter. We found that stroke induces diverse changes in the expression profiles of individual Class I and Class II HDACs in OPCs and OLGs.

2. Material and methods

All experimental procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee (IACUC) of Henry Ford Health System. All efforts were made to minimize suffering of animals.

2.1. Animal model of middle cerebral artery occlusion (MCAO)

Adult male Wistar rats (3–4 months, 270–300 g) were employed in all experiments. The middle cerebral artery (MCA) was permanently occluded via an intraluminal vascular occlusion method modified in our laboratory (Chen et al., 1992; Zhang et al., 2009). Briefly, following anesthesia and neck dissection, a 4–0 surgical nylon suture (18.5–19.5 mm) determined by the animal weight, with its tip expanded by heating near a flame, was advanced from the right external carotid artery (ECA) into the lumen of the internal carotid artery (ICA) to block the origin of the MCA. Rats (n = 6–8/group) were subjected to MCAO and sacrificed at 2, 7 or 14 days after stroke. Sham-operated rats were similarly anesthetized and neck dissection performed without suture advancement.

2.2. Bromodeoxyuridine labeling

Bromodeoxyuridine (BrdU), a thymidine analog that is incorporated into cells during DNA synthesis, was used for S-phase labeling. Using a cumulative labeling protocol (Zhang et al., 2001), rats (n = 6–8/group) were injected daily with BrdU (Sigma–Aldrich, St. Louis, MO), intraperitoneally at 50 mg/kg/day for 2 or 7 consecutive days, starting 24 h after surgery. Animals were sacrificed 2 or 7 days after surgery at 2 h after the last BrdU injection. Animals sacrificed at 14 days after surgery were injected daily with BrdU for 7 days starting 24 h after surgery.

2.3. Tissue preparation

Brains were fixed by transcardial perfusion with saline, followed by perfusion and immersion in 4% paraformaldehyde. Brains were cut into seven equally spaced (2 mm) coronal blocks and embedded in paraffin. A series of coronal sections (6 μm thick) were obtained at the center of the lesion, corresponding to coronal coordinates for Bregma −1 to +1 mm (Paxinos and Watson, 1986), and used for immunohistochemistry.

2.4. Immunohistochemistry

Immunohistochemistry was performed following standard protocols, as previously described (Liu et al., 2012). The following primary antibodies were used: mouse anti-Adenomatous Polyposis Coli (APC [CC-1], 1:20; GWB-D835F1, GenWay, San Diego, CA), a marker for mature OLGs (Bhat et al., 1996), mouse anti-Chondroitin Sulfate Proteoglycan (NG2, 1:1000; 05–710, Millipore, Billerica, MA), a marker of OPCs (Ness et al., 2005), mouse anti-BrdU (1:100; M0744, Dako, Carpinteria, CA) and rabbit anti-Ki67 (1:300; RM-9106, Thermo Scientific, Barrington, IL), markers for proliferating cells. Class I and Class II HDACs were examined using: rabbit anti-HDAC1 (1:2000; ab7028, Abcam, Cambridge, MA), rabbit anti-HDAC2 (1:250; sc-7899, Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-HDAC4 (1:50; sc-11418, Santa Cruz Biotechnology) and rabbit anti-HDAC5 (1:50; sc-11419, Santa Cruz Biotechnology).

Histone acetylation levels were examined using: rabbit anti-Acetyl Histone H3 Lys9/Lys14 (Ac-HH3, 17–615, 1:1200; Millipore)