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Role of HDACs in optic nerve damage-induced nuclear atrophy of retinal ganglion cells

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Abstract

Optic neuropathies are characterized by retinal ganglion cell (RGC) death, resulting in the loss of vision. In glaucoma, the most common optic neuropathy, RGC death is initiated by axonal damage, and can be modeled by inducing acute axonal trauma through procedures such as optic nerve crush (ONC) or optic nerve axotomy. One of the early events of RGC death is nuclear atrophy, and is comprised of RGC-specific gene silencing, histone deacetylation, heterochromatin formation, and nuclear shrinkage. These early events appear to be principally regulated by epigenetic mechanisms involving histone deacetylation. Class I histone deacetylases HDACs 1, 2, and 3 are known to play important roles in the process of early nuclear atrophy in RGCs, and studies using both inhibitors and genetic ablation of *Hdacs* also reveal a critical role in the cell death process. Select inhibitors, such as those being developed for cancer therapy, may also provide a viable secondary treatment option for optic neuropathies.

Keywords

Histone deacetylase (HDAC); neurodegeneration; retinal ganglion cell; nuclear atrophy; heterochromatin; apoptosis; optic nerve injury; glaucoma

1. Introduction

Optic neuropathies, such as glaucoma, result in retinal ganglion cell (RGC) death. In glaucoma, increased intraocular pressure culminating in increased strain at the optic nerve head is thought to induce axonal damage leading to further pathology in this disease. Axogenic neurodegeneration can be mimicked in rodent models of acute optic nerve injury. Optic nerve crush (ONC) in rodents has been used extensively to model the pathophysiology to RGCs [1]. The sequence of apoptotic events has been described in this model, and includes early onset nuclear atrophy [2–4]. Global and focal changes in the chromatin of RGCs occur shortly after acute injury of the optic nerve and before the cell has reached the

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committed step of intrinsic apoptosis, pro-apoptotic BAX oligomerization at the mitochondrial outer membrane [2]. Epigenetic modification of histones, principally their deacetylation, has been implicated in not only early nuclear changes in RGCs, but also in their apoptotic death.

Histone deacetylases (HDACs) remove acetyl groups from lysine residues. In the nucleus, deacetylation of histones is involved in chromatin condensation, guiding gene expression during development and differentiation, and neuronal death [5, 6]. HDACs are grouped into four classes based on phylogenetic analysis [6]. Class I includes HDACs 1, 2, 3, and 8; class II includes HDACs 4, 5, 6, 7, 9, and 10; Class III includes SIRT1s 1, 2, 3, 4, 5, 6, and 7; and class IV includes HDAC11 [6]. There is growing evidence that HDAC activity is a critical component of neuronal death, and is implicated in animal models of Huntington's disease, stroke and ischemic injury, spinocerebellar ataxia type 7, and memory loss in cocaine-seeking behavior [7–10]. More recently, HDAC activity has been associated with the loss of retinal ganglion cells (RGCs) in models of optic nerve damage.

The advent of new and selective inhibitors of these enzymes, suggests an intriguing opportunity to target HDACs as a therapeutic strategy for neurodegenerative conditions. Here, we discuss the current state of knowledge of the roles of HDACs in the process of neuronal, and specifically, retinal ganglion cell degeneration.

2. HDACs regulate chromatin remodeling during nuclear atrophy in RGCs

HDACs 1, 2, 3, 5, and 6 have all been identified in the murine retina previously [3, 11]. In healthy RGCs, HDACs 1 and 2 are localized to the nuclei while HDAC3 is localized mainly to the cytoplasm [3, 11]. The optic nerve crush injury model has been used extensively to study HDAC activity in the process of RGC death. In this model, intrinsic apoptosis is activated leading to a loss of neurons beginning around 7 days after injury. After acute optic nerve injury, mRNA accumulation of class I HDACs increases, with *Hdac2* and *Hdac3* expression being the highest at 3 days post ONC [3]. HDAC3 translocates from the cytoplasm to the nucleus of RGCs by 3–5 days following optic nerve damage [3, 4]. These observations are consistent with other studies where HDAC3 is cytoplasmic in healthy neurons, but localized to the nuclei of diseased cortical neurons of a mouse model of Huntington's disease [9].

Heterochromatin formation is a signature feature of apoptosis [12, 13]. It initiates along the inner surface of the nuclear envelope and coalesces throughout the nucleus in a process defined as pyknosis. The nuclear envelope is also broken down and the condensed chromatin is fragmented and often detected in apoptotic bodies. DNA fragmentation assays, such as TUNEL [14], show that pyknosis is associated with DNA cleavage. The responsible endonuclease for this process is activated in a caspase-dependent manner [15]. Dying RGCs undergo pyknosis, but studies using *Bax*-deficient mice, in which the apoptotic pathway is blocked before caspase activation, suggest that chromatin remodeling is initiated well in advance of DNA cleavage [2]. We have documented several metrics of this process in RGCs, including heterochromatin formation and silencing of RGC-specific gene expression [2–4, 16]. All of these processes occur in *Bax*-deficient cells, and are related to HDAC activity.

Silencing of genes selectively expressed by RGCs occurs within 24 hours post optic nerve injury in rodents [3, 17]. Chromatin immunoprecipitation studies showed that the acetylation state of histone H4 associated with promoters of several RGC-specific genes was significantly reduced by 24 hours following ONC in the mouse [3]. Additionally, treatment of mice with the broad-spectrum inhibitor trichostatin A (TSA) was able to attenuate the silencing of many of these genes [3, 16]. Taken together, these data provide compelling evidence of a role of HDAC activity in this process. Which HDACs participate in this process is uncertain. Selective ablation of *Hdacs* in RGCs using conditional knockout mice has been used to address this question. Gene excision is accomplished by transducing RGCs with adeno-associated virus serotype 2, which has a high tropism for RGCs [18], carrying the CRE recombinase. Interestingly, genetic ablation of *Hdac3* in mouse RGCs was unable to prevent silencing of the target genes [4], while double knockout of *Hdac1* and *Hdac2* had a similar lack of effect on the overall pattern of down-regulated genes when assessed by RNA sequencing [19]. Thus studies using inhibitors or genetic ablation do not reconcile. It is possible that genetic deletion experiments are complicated by other HDACs playing a compensatory role and it remains unclear how HDACs participate in the mechanism of gene silencing.

While gene silencing may involve focal changes in histone deacetylation, the process of global heterochromatin formation appears to be associated with widespread deacetylation. After optic nerve injury, RGC nuclei exhibit dramatic and widespread deacetylation of histones, a process that is initiated early and continues to a maximum decrease by 5 days (Figure 1) [3]. Unlike the process of gene silencing, global deacetylation is clearly attributable to the function of HDAC3. Mice lacking *Hdac3* in RGCs exhibited nearly complete abrogation of this response, which was, in turn associated with a significant reduction in the formation of heterochromatin (Figure 1) [4].

Given the evidence currently available, we posit that RGC apoptosis is associated with at least two phases of histone deacetylation. The first appears to be rapid and focally restricted to areas of active gene expression, while the second is more prolonged and widespread. This latter phase may be specific to the later stages of nuclear atrophy as a prelude to the activation of catabolic pathways that lead to DNA fragmentation.

3. HDAC inhibition prevents RGC death

HDAC activity modulates changes in chromatin structure and influences the gene expression profile of damaged RGCs. It is not known if these changes directly precipitate downstream apoptotic events. The importance of HDAC activity in the process of RGC death was initially interrogated using broad-spectrum HDAC inhibitors, which exhibit neuroprotective effects in a wide range of models of neurodegeneration [20]. Rats treated with the class I and II HDAC inhibitor valproic acid (VPA), had significantly less RGC loss by 8 days after acute optic nerve injury than vehicle treated rats [21]. Similarly, treatment of purified rat RGCs with VPA or sodium butyrate (SB), another class I and class IIa HDAC inhibitor, prevented histone deacetylation and protected RGCs from death *in vitro* [22]. TSA was similarly protective in mouse models of acute optic nerve damage [3] and acute hypertension induced retinal ischemia [11]. More selective inhibition of HDACs 1, 2, and 3 with MS-275,

protected ganglion cells as long as 6 weeks following optic nerve injury [23]. Inhibitors are also reportedly protective in rodent models of experimental glaucoma. Treatment with VPA in a rat model of ocular hypertension helped preserve retinal function as assessed by electroretinograms, and reduced the rate of RGC loss [24]. TSA attenuated progressive RGC soma degeneration in the DBA/2J mouse model of glaucoma, but had no significant effect on axonal degeneration [16]. Interpreting results obtained with inhibitors, particularly broad spectrum ones, may be complicated by the indiscriminate nature in which they act. Prevention of HDAC activity in the RGC nucleus, for example, may be protective, but preventing deacetylation of non-histone targets may be detrimental. The development of more selective inhibitors may greatly advance both the understanding of the roles of different HDACs, while providing a more granular therapeutic approach that selectively targets pathologic HDACs and preserves the vital activities of others.

4. Class I HDACs 1, 2, and 3 regulate apoptosis in differentiated neurons

The experiments using HDAC inhibitors, reviewed above, indicate that blocking HDAC functions can have a profound protective effect on the loss of not only RGCs, but also differentiated neurons in general, in a variety of neurodegenerative conditions. While some of the consequences of HDAC activity may not be directly lethal to cells, RGCs, for example can tolerate heterochromatic chromatin for 18 months if the apoptotic program is blocked [25], their actions can clearly precipitate further downstream events that lead to cell death. A clue to these effects were demonstrated by experiments by Bardai and D'Mello [26], who showed that forced expression of HDAC3 promoted the death of hippocampus-derived HT22 cells, and rat cortical neurons, but not primary kidney fibroblasts, or HEK293 and HeLa cell lines. In neurons, HDAC3 is activated by phosphorylation from GSK3 β serine/threonine kinase (Figure 2): a mechanism that is normally inhibited by growth factor-stimulation of the PI3K/Akt signaling pathway [26]. The selectivity of HDAC3 expression to induce the death of neurons is not well understood, but it may be mediated through the activation of the tumor suppressor protein p53. Deacetylation of p53 can activate its function as a transcription factor either by promoting nuclear accumulation [27] or enhancing binding to p53 response elements on target genes [28], or both. In neurons, deacetylation of lysine residues K381 and K382 (human p53), appear to be critical for activating pro-apoptotic gene expression [28], while other studies have identified K317 (mouse p53) as being important for the same function [29]. RGCs in mice exhibit significant deacetylation of residue K381 within a few days after optic nerve injury, a process that is attenuated in cells lacking both *Hdac1* and *Hdac2* [19].

Once activated, p53 directs the transcription of a variety of genes, including those involved in cell-cycle checkpoints and others that induce apoptosis. Importantly, in this latter category, p53 induces the expression of activator proteins containing the BH3 homology domain from the larger BCL2 gene family, including *Bbc3* and *Bim*, which lead directly to the activation of latent BAX in cells (Figure 2). Why HDAC3 is selective toxicity to neurons is not well understood, but promoter targeting of p53 is regulated differentially, depending on whether or not the cell is actively dividing [30]. The p53 response elements are more highly conserved in cell cycle regulatory genes than in the promoters of pro-apoptotic genes [31]. In addition, p53 preferentially targets cell cycle regulatory genes in DNA binding

assays, regardless of the stress conditions [32]. Neurons, however, may have a propensity to target p53 to pro-apoptotic genes such as *Bbc3* and *Bim*. This process that may be influenced by early apoptotic changes in chromatin structure that suppress cell-cycle regulatory p53 targets [33]. Consistent with this, HDAC2 activity in cortical granular neurons was found to be associated with the inhibition of expression of potentially protective genes. HDAC2 associates with the transcription factor FOXO3_a directing it to the promoter region of the cell cycle arrest gene *p21*, where it suppressed *p21* expression by hypoacetylating the promoter (Figure 2) [34]. Knockdown of *Hdac2* in these cells [34] or pulse inhibition with HDAC inhibitors [35] was protective after exposure to oxidative stress and tied directly to the up-regulation of *p21* expression. Paradoxically, *Hdac1/2* deletion in RGCs reduces *p21* expression after optic nerve damage [19], suggesting a more complex interaction that still needs to be resolved.

5. HDAC isoform targeting as a potential therapeutic for glaucoma

Since the treatment of glaucoma is currently limited to lowering intraocular pressure, there is a concerted effort to find adjunctive therapies that are directed specifically at the tissues, which are principally affected. To date, broad spectrum HDAC inhibitors have shown great promise as therapeutics in animal models of RGC death, however further interrogation of these and more selective inhibitors is necessary for eventual treatment of optic neuropathies such as glaucoma. RGC death in glaucoma occurs over time, and is often asynchronous and protracted. Given the chronic nature of glaucomatous disease, treatment administration would be necessary over months or years to prevent vision loss in patients who have been diagnosed. High doses and extended administration of broad spectrum HDAC inhibitors may have toxic effects on other cell types such as proliferative cells and immune cells [36, 37], and importantly, use of high doses of VPA, SB, or TSA is actually toxic to primary RGCs [22]. It has been suggested that use of HDAC isotype selective inhibitors may avoid potentially harmful side effects when attempting to specifically treat degenerating neurons [38]. An advantage in treating optic neuropathies, however, may be to locally administer HDAC isotype selective inhibitors using intravitreal injection or other adapted methods, which would reduce the potentially toxic effects of prolonged systemic administration of HDAC inhibitors. Protocols using monthly intravitreal injections of VEGF inhibitors are now standard practice for macular degeneration, so similar protocols to treat glaucoma may be feasible.

6. Conclusion

While the exact biological mechanisms of each individual HDAC are not fully elucidated in injured RGCs, investigations in these cells and other neurodegenerative disease models shed light on the varying pathways that HDACs regulate. Since inhibition of HDACs promotes both neuronal survival and cancer cell apoptosis, HDAC inhibitors are widely used for study and some are already in clinical trials [8, 10, 36, 39–42]. It is important to recognize that these inhibitors, especially broad-spectrum inhibitors, are toxic to rapidly dividing cells such as immune cells and intestinal cells when administered at high doses [36, 37]. The role of HDACs in nuclear atrophy and apoptosis of RGCs must be further investigated to determine

which HDACs serve as appropriate targets for therapeutic inhibition in optic neuropathies such as glaucoma.

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HIGHLIGHTS

- HDACs 1, 2, and 3 play major roles in retinal ganglion cell (RGC) death.
- Nuclear atrophy is associated with increased HDAC activity in RGCs
- HDAC3 is selectively toxic to neurons.
- HDAC inhibition may yield new therapeutic options to treat optic neuropathies.

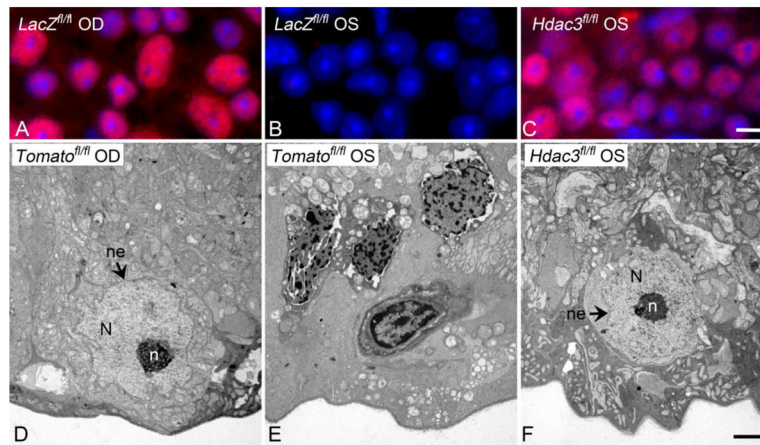


Figure 1. Conditional knockout of *Hdac3* prevents heterochromatin formation after optic nerve crush

Conditional knockout of *Hdac3* in RGCs was achieved by injecting AAV2-Cre virus into the left (OS) eye of *Hdac3^{fl/fl}* mice prior to ONC. AAV2-Cre injected into the OS eyes of *Rosa26-Tomato^{fl/fl}* mice served as virus-injected controls. Contralateral right (OD) eyes served as uncrushed and non-injected controls. Retinas were harvested at 5 days following crush injury for evaluation. (A) Retinal whole mount showing staining for acetylated histone H4 in unaffected OS eyes. (B) Crush elicits wide spread deacetylation of histones, but this process is blocked in RGCs lacking *Hdac3* (C). DAPI counterstain. (Scale bar = 10 μ m). (D) Transmission electron micrograph (TEM) image of a healthy cell in the ganglion cell layer (GCL) of a *Rosa26-Tomato^{fl/fl}* control OD eye. (E) TEM image of heterochromatic cells in the GCL of an AAV2-Cre/GFP injected and crushed *Rosa26-Tomato^{fl/fl}* OS eye. (F) TEM image of a cell in the GCL of the *Hdac3* conditional knockout crushed OS eye. Healthy nuclei (N) are euchromatic and have well-formed nucleoli (n) and intact nuclear envelopes (ne), while injured cells exhibit heterochromatic nuclei with degrading nuclear envelopes. (Scale bar = 2 μ m)

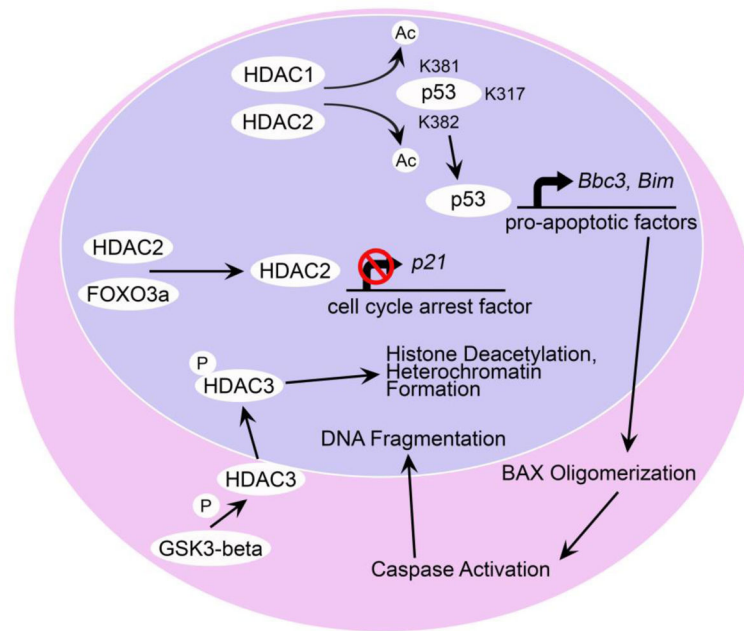


Figure 2. Potential pathways of HDAC activity in neuronal toxicity

HDAC1 and HDAC2 reportedly activate p53 by deacetylating its K381 and K382 residues, where it leads to an increase in the expression of genes involved in apoptosis, including, *Bbc3* (PUMA), and *Bim* [27–30]. These BH3-only activator proteins then facilitate the activation of BAX, leading to caspase activity and the activation of an endonuclease that digests DNA. HDAC2 may have an additional function in silencing other p53-target genes, such as p21. In this mechanism, HDAC2 is associated with FOXO3_a, which recruits it to the *p21* promoter site, where it deacetylates histones in this region and represses transcription [34]. HDAC3 plays a primary role in histone deacetylation, and heterochromatin formation [3, 4]. In neurons, HDAC3 is activated by phosphorylation from GSK3 β serine/threonine kinase, which becomes active when the PI3K/Akt signaling pathway is attenuated by loss of trophic factor support [26].