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# Therapeutic Approaches to Histone Reprogramming in Retinal Degeneration

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## Abstract

Recent data have revealed epigenetic derangements and subsequent chromatin remodeling as a potent biologic switch for chronic inflammation and cell survival which are important therapeutic targets in the pathogenesis of several retinal degenerations. Histone deacetylases (HDACs) are a major component of this system and serve as a unique control of the chromatin remodeling process. With a multitude of targeted HDAC inhibitors now available, their use in both basic science and clinical studies has widened substantially. In the field of ocular biology, there are data to suggest that HDAC inhibition may suppress neovascularization and may be a possible treatment for retinitis pigmentosa and dry age-related macular degeneration (AMD). However, the effects of these inhibitors on cell survival and chemokine expression in the chorioretinal tissues remain very unclear. Here, we review the multifaceted biology of HDAC activity and pharmacologic inhibition while offering further insight into the importance of this epigenetic pathway in retinal degenerations. Our laboratory investigations aim to open translational avenues to advance dry AMD therapeutics while exploring the role of acetylation on inflammatory gene expression in the aging and degenerating retina.

### Keywords

Retinal degeneration; Acetylome; Lysine deacetylases; Histone deacetylases; Valproic acid; Apoptosis; Inflammation; Aging Electronic supplementary material

## 6.1 Post-translational Acetylation Controls Gene Expression and Protein Activity

Acetylation is a reversible post-translational modification that was first discovered in histones and occurs in a wide range of organisms. Histone proteins (H2A, H2B, H3 and H4) are integrated with 147 base-pairs of DNA in a complex called the nucleosome (Luger et al. 1997). Lysine acetylation and deacetylation of histones are carried out by two groups of

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enzymes: acetyl group addition by HATs (or lysine acetyltransferases KATs) and acetyl group removal by HDACs (or KDACs), respectively. A generalized epigenetic principle is that histone acetylation results in an open structure of the DNA enabling gene transcription whereas histone deacetylase activity tightens the nucleosome and compacts the chromatin making those sites inaccessible for transcription (de Ruijter et al. 2003). While this model has not applied to all systems, it is clear from current studies that there is a delicate balance of (de)acetyltransferase activity which may be dysregulated in aging diseases. Lysine acetylation is not limited to histones but are also present in innumerous other protein substrates giving acetylation a wider significance in developmental and disease states (Peserico and Simone 2011). Nuclear receptors (estrogen receptor, p300), proliferating factors (E2F/RB), hypoxia induced factors (HIF-1α), transcription factors (NFκB, p53, STAT3 and c-MYC) and other cellular proteins (αTubulin, Ku70 and Hsp90) are all known non-histone targets of HATs and HDACs (Glozak et al. 2005).

Histone deacetylases are a family of 18 known members, classified in four groups based on their homology to yeast proteins (Dokmanovic et al. 2007). Class I consists of HDAC1, 2, 3 and 8. HDAC1 and HDAC2 are ubiquitously expressed, strongly localized to nuclei and predominantly associated in megadalton complexes (Bantscheff et al. 2011; Di Marcotullio et al. 2011). Members of the Class II-family of HDACs are separated into Class IIa (HDAC4, 5, 7 and 9) which localize to both nuclear and cytosolic compartments and IIb (HDAC6 and 10) which are predominantly cytosolic. Class I/II HDACs are zinc dependent. Class III HDACs, also known as sirtuins (SIRTs), are evolutionarily unrelated to the other HDAC classes. SIRTs require $NAD^+$ as a co-factor making them highly sensitive to oxidative stress (Balaiya et al. 2012) and a hotly pursued potential therapeutic target for age-related and metabolic diseases (Imai and Guarente 2014). There is only one Class IV HDAC (HDAC11) which is also zinc dependent, localized to the nucleus and is heavily conserved in all living eukaryotes other than fungi (Gao et al. 2002).

## 6.2 Context and Tissue Dependent Effects of Histone Deacetylases

Expression levels of HATs and HDACs as well as targeted acetylation sites and proteins can dramatically change between tissues and within varying developmental, normal adult, and diseased states. There are several models of neuronal cell death in which HDAC inhibition (HDACi) exhibits a protective benefit whereas targeting identical pathways in cancer cells is pro-apoptotic. With the highly specialized and multicellular architecture of the eye, the dominant effects of HDAC inactivity remain very unclear and certainly become extremely difficult to interpret when comparing acute laboratory animal and cell culture models to chronic aging diseases. Still, we are gathering essential data that will allow us to develop a fundamental understanding of this critical and deeply conserved regulatory biologic system. Class I HDAC expression is considered ubiquitous though we have observed significant differences in immuno-localization patterns in the mouse retina with HDAC1/2. Class II HDACs are known to display highly specific tissue-dependent expression patters leading to variable sub-cellular localization and certainly tissue-specific biological effects of enzyme inactivity and subsequent imbalances in the acetylome. Many diseases have been associated with altered global acetylation patterns including cancer, cardiovascular disease and inflammatory diseases. Hyper-acetylation via HDA-Ci is known to be cytoprotective in

models of neuronal ischemic injury (Kim and Chuang 2014; Murphy et al. 2014), Huntington's disease (Ferrante et al. 2003), and stroke (Liu et al. 2012). Yet, HDACi is nearly uniformly cytotoxic in cancer models (McConkey et al. 2012). HDACi also has opposing effects on critical immune system mediators. Toll-like receptors (TLRs) are potent cell-signaling gateways to innate immune pathways and downstream inflammatory responses. Treatment of cultured human macrophages with HDACi leads to caspase-dependent apoptosis and release of pro-inflammatory cytokines; however, this effect is reversed by pre-treatment with TLR agonists including LPS and poly I:C (Tsolmongyn et al. 2013). Data revealing the protean biology of the acetylome must be seriously addressed and rigorously studied in the laboratory prior to pharmacologically approaching HDAC/HAT manipulation for the treatment of human diseases.

A potent and well-characterized Class I/II HDACi is suberoylanilide hydroxamic acid (SAHA also known as vorinostat) which is currently in advanced phase clinical phase trials for multiple myeloma and several solid tumors. Similar to previous HDACi results, the pharmacologic effects of SAHA are highly dependent on cell-type and state coupled with a limited therapeutic window. While low concentrations of SAHA may be significantly cytoprotective, higher concentrations are pro-apoptotic in many immune cell types (Li et al. 2008). Nearly identical data exists for valproic acid (VPA), another small molecule Class I/II HDACi which is most widely used for seizure prophylaxis. VPA has been shown to reduce brain damage in an animal model of transient cerebral ischemia (Ren et al. 2004), provide acute neuro-protection in ischemic retinal injury (Alsarraf et al. 2014), and stimulate axonal regrowth after optic nerve crush (Biermann et al. 2010). These data have rapidly opened translational avenues of pharmacologic induced chromatin remodeling as a novel target for the epigenetic regulation of critical cell death and survival pathways in aging and neurodegenerative diseases.

## 6.3 Differential Effects of Histone Deacetylase in the Retina and Retinal Pigment Epithelium

Investigations of the *rd1* mouse demonstrated significant protection form loss of photoreceptors after broad inhibition of Class I/II HDACs with trichostatin A (TSA) (Sancho-Pelluz et al. 2010). A single report was then published suggesting the therapeutic efficacy of VPA in the treatment of retinitis pigmentosa (Clemson et al. 2011). Similar benefits were reported for VPA in *rd1* mice (Mitton et al. 2012); however, the same treatment had the contrary effect in *rd10* mice (Guzman et al. 2014). Additional studies were performed even though the original data had been hotly contested with multiple letters in the literature with severe limitations to the study design and reports of inefficacy and even loss of vision associated with the use of VPA for retinal degeneration (van Schooneveld et al. 2011; Sisk 2012). Recently, a long-term follow-up study confirmed visual decline and adverse side-effects associated with VPA therapy in patients with retinitis pigmentosa (Bhalla et al. 2013).

Four independent groups have presented varying data regarding VPA and retinal degeneration. Reports included positive (Iraha et al. 2014), variable (Guzman et al. 2014;

Lai et al. 2014) or outright negative findings (Berner et al. 2014; Kumar et al. 2014). In a transgenic *Xenopus* model expressing various human rhodopsin mutations, only retinal degeneration secondary to the P23H mutation was favorably treated with VPA (Lai et al. 2014). Despite VPA's described neuro-protective and anti-inflammatory properties, in just these few studies, significant retinotoxicity was encountered in numerous animal and cell-culture models. We have demonstrated that VPA up-regulates caspase-3 activation and cell death in primary human RPE isolates, a finding which has been confirmed in other studies (Suuronen et al. 2007; Kumar et al. 2014). VPA treatment exhibits a significant pro-inflammatory response *in vitro* and *in vivo* with an array of cytokines, cytokine receptors, mediating enzymes and transcription factors (Kleinman et al. 2013; Kleinman et al. 2014). This pro-inflammatory signature is in accordance to the known immune response in AMD (Suuronen et al. 2007; Shakespear et al. 2011; Miao et al. 2012; Whitcup et al. 2013). Further investigations into this powerful epigenetic regulatory system will continue to yield important features of HDAC involvement in the pathogenesis and treatment of retinal degenerations; however, at this time we urge caution using VPA as a treatment option for these diseases given the variable treatment effect dependent on tissue-type and cellular target (Fig. 6.1).

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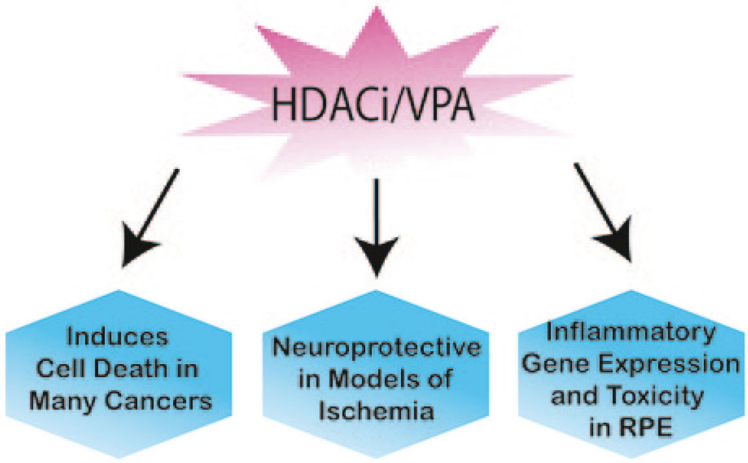


**Fig. 6.1.**

While HDAC inhibition is cytoprotective in many models of neuronal cell death, it is also capable of inducing significant cytotoxicity in various cancers and up-regulating inflammatory gene expression and cell death in the RPE