# Chapter 61
# Histone Deacetylase: Therapeutic Targets in Retinal Degeneration

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**Abstract** Previous studies report that retinitis pigmentosa (RP) patients treated with the histone deacetylase inhibitor (HDACi) valproic acid (VPA) present with improved visual fields and delayed vision loss. However, other studies report poor efficacy and safety of HDACi in other cohorts of retinal degeneration patients. Furthermore, the molecular mechanisms by which HDACi can improve visual function is unknown, albeit HDACi can attenuate pro-apoptotic stimuli and induce expression of neuroprotective factors. Thus, further analysis of HDACi is warranted in pre-clinical models of retinal degeneration including zebrafish. Analysis of HDAC expression in developing zebrafish reveals diverse temporal expression patterns during development and maturation of visual function.

**Keywords** Histone deacetylase · Histone deacetylase inhibtors · Retinal degeneration · Retinitis pigmentosa · Zebrafish

**Abbreviations**

| | |
|---|---|
| BDNF | Brain derived neurotrophic factor |
| CNTF | Ciliary neurotrophic factor |
| DPF | Days post fertilisation |
| HAT | Histone acetyltransferase |
| HDAC | Histone deacetylase |
| HDACi | Histone deacetylase inhibitor |
| HPF | Hours post fertilization |
| *rd1* | Retinal degeneration 1 |

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RP Retinitis pigmentosa
TSA Trichostatin A
VA Visual acuity
VF Visual field

## 61.1 Introduction

The 18 HDAC proteins are divided into two families, "classical" HDACs and SIR2 HDACs which are further subdivided into four classes based on homology to yeast HDAC orthologues and functional activity. In general, Class I members (HDAC1, 2, 3, 8) are localized to the nucleus while Class II members (HDAC4, 5, 6, 7, 9, 10) can be either localised in the nucleus or cytoplasm. Class III are a family of 7 NAD+ dependent proteins, known as sirtuins (SIRT1–7), similar to yeast Sir2 proteins. Class IV HDACs show structural similarity to both Class I and II HDACs (Yang and Seto 2008). These proteins can control gene transcription via epigenetic alteration of chromatin or modulate the activity of non-histone proteins by altering their acetylation (Choudhary et al. 2009). Consequently, HDACs regulate cell cycle progression, differentiation and survival.

## 61.2 HDACi as Potential Therapeutics for Treatment of Retinal Degeneration

A retrospective study of 7 RP patients reported improved visual field (VF) and visual acuity (VA) scores and delayed vision loss in five patients following treatment for 4 months with a mean dose of 643(+/− 133) mg/day valproic acid (VPA) (Clemson et al. 2011). Only mild side-effects, such as fatigue and stomach irritation were reported and liver function and blood chemistry remained normal. However, in a similar study of pigmentary dystrophy patients treated with 500–1000 mg/day VPA for 10 months; the five patients for which VF field tracings were available before and after treatment presented with a decline in VF, 22 patients had a decline in VA and 12 patients reported severe negative side effects inclding high alanine aminotransferase, aspartate aminotransferase and ammonia levels (Bhalla et al. 2013). The Clemson study has been criticised regarding study design, patient numbers (van Schooneveld 2011), and statistical analyses (Sandberg et al. 2011). Indeed, VPA may compromise photoreceptor function due to antagonistic effects on sodium and calcium channels in the retina (Sisk 2012). Despite these concerns, a randomized, placebo-controlled trial of oral VPA for treatment of autosomal dominant RP (NCT01233609), and a non-randomized trial (NCT01399515) are in progress.

## 61.3 HDAC Inhibition in a Pre-Clinical Rodent Model of Retinal Degeneration

In the *rd1* (retinal degeneration 1) mouse model of RP, histone acetylation is dramatically reduced in retinal cells. Retinal degeneration in *rd1* mice is mediated by phosphodiesterase-6 (PDE6) dysfunction resulting in high cyclic guanosine-monophosphate (cGMP) levels and increased oxidative stress (Sahaboglu et al. 2013). Increased expression of cell proliferation and oxidative stress genes is observed during *rd1* photoreceptor degeneration (Hackam et al. 2004) as is increased HDAC activity, with class I/II HDACs contributing the majority of total HDAC activity (Sancho-Pelluz et al. 2010). TUNEL positive cells in the degenerating *rd1* mouse eye also have reduced histone acetylation. Overall, reduced histone acetylation due to aberrant HDAC activity appears to be a major contributing factor to retinal degeneration in the *rd1* model. Notably, treatment of *rd1* retinal explants with Class I/II HDAC inhibitors, 1 μM Trichostatin A (TSA) or 6 μM Scriptaid, reduced photoreceptor cell death and restored photoreceptor outer segments (Sancho-Pelluz et al. 2010). These results suggest a major contribution of class I/II HDACs, to mutation-induced *rd1* photoreceptor cell death.

## 61.4 Mechanism of Action

A number of mechanisms by which HDACi produce their therapeutic effects have been suggested. HDACi diminish the activity of the Hsp90 chaperone, by increased acetylation (Scroggins et al. 2007; Kekatpure et al. 2009). Hsp90 inhibition increases expression of the neuroprotective chaperone Hsp70, which promotes neuronal survival (Wen et al. 2008). TNF-α is lowly expressed in wildtype retina but increased in models of ischemic injury (Genini et al. 2013). Pharmacological inhibition of Class I/II HDACs with 2.5 mg/kg TSA blocks increases in TNF-α levels in the rat eye post ischemic injury (Crosson et al. 2010). HDACi also modulate expression of brain derived neurotrophic factor (BDNF) via repression of its promoter. Selective pharmacological inhibition of class II HDACs with 5 μM MC1568 leads to rapid induction of BDNF expression while inhibition of class I HDACs with 5 μM MS-275 leads to a comparatively slower induction (Koppel and Timmusk 2013). In agreement, treatment of *rd1* retinal explants with BDNF and ciliary neurotrophic factor (CNTF) provides a neuroprotective effect (Azadi et al. 2007).

## 61.5 HDAC Expression in The Zebrafish Model

Zebrafish eye development is rapid. At 11 hpf the optic vesicle is visible (Kimmel et al. 1995). At 3 days post fertilisation (dpf) all cell types of the retina have differentiated and measurable cone mediated visual responses develop (Easter and

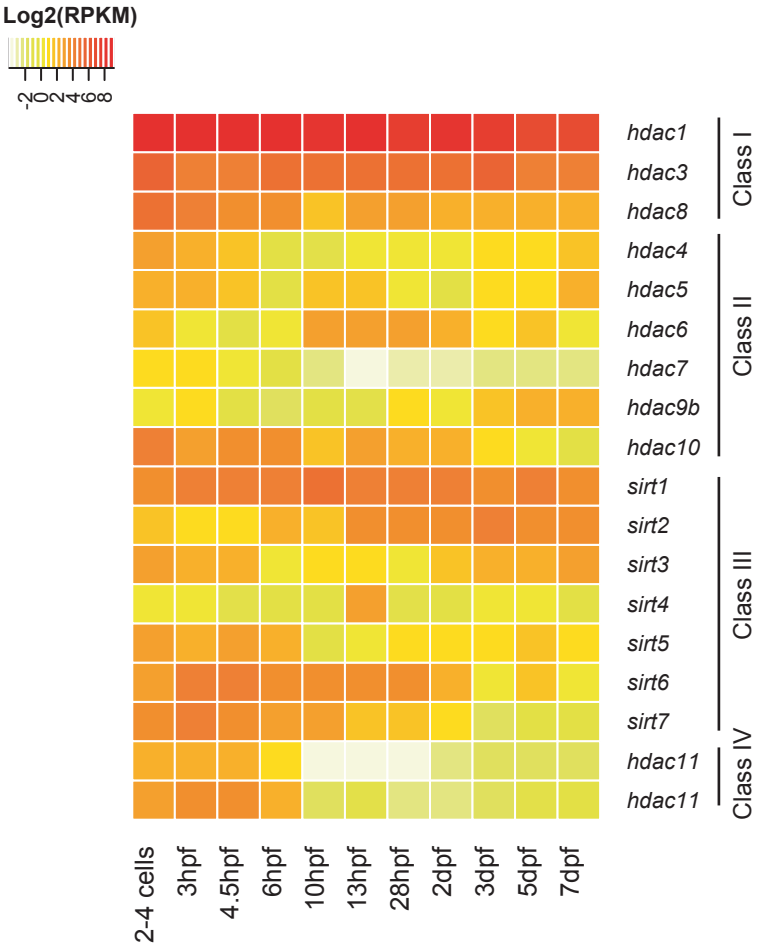


**Fig. 61.1** Heatmap overview of gene expression profiles of HDACs using RNA-sEq. RNA-seq data sets on whole embryos were used. Genes expression levels were depicted using Log2 transformed Reads per kilobase per million (*RPKM*)

Nicola 1996). The zebrafish eye has a similar structure to other vertebrates, sharing the cell types and laminate structure present in humans. In early stages of development (2–16 hpf) *hdac1* is ubiquitously expressed. At later stages (36–48 hpf), expression is partially restricted to the branchial arches, fin bud mesenchyme and hindbrain. Pharmacological inhibition of HDACs by TSA results in a failure of craniofacial cartilage to develop from these tissues (Pillai et al. 2004). Similarly, in the hindbrain of *hdac1* mutants there is reduced cell proliferation marked by defects in axial extension of hindbrain branchiomotor neurons caused by reduced activation of non-canonical Wnt/PCP pathway regulators (Cunliffe 2004). In addition, inhibition of class I/II HDACs affects migration of the posterior lateral line primordium. Treatment disrupted neuromast deposition in a dose dependent

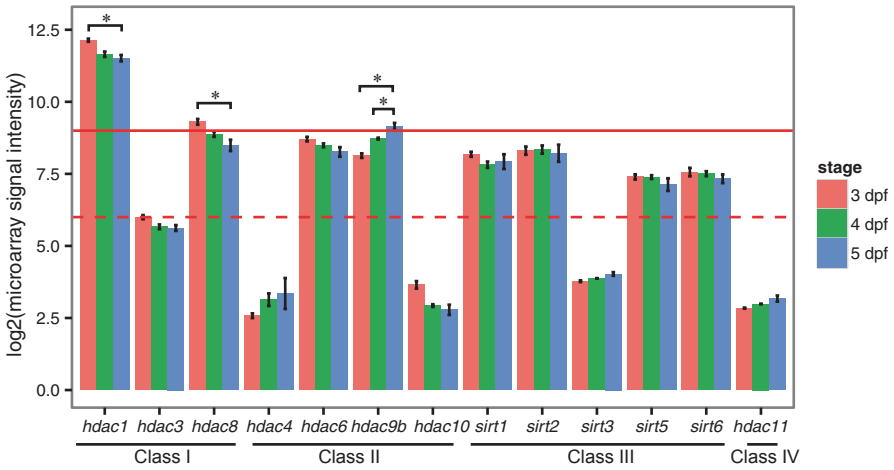


**Fig. 61.2** Gene expression profiles of HDACs on microarray. Log2 transformed signal intensities of embryonic eyes on 3, 4 and 5 days post fertilization (*dpf*). The *solid red line* indicates high gene expression (log2 signal intensity of 9). The *dashed red line* indicates medium gene expression (log2 signal intensity of 6). **p*-value < 0.05

manner (He et al. 2014). Treatment with VPA also reduces proliferation of neural stem cells in the adult zebrafish optic tectum via inhibition of Notch signaling (Dozawa et al. 2014).These reports underline the importance of HDAC activity for cell proliferation and migration.

An analysis of publically available RNA-seq data (Fig. 61.1) depicts the expression of zebrafish HDACs during development in whole larvae (Aanes et al. 2011; Collins et al. 2012). *Hdac* genes show diverse expression patterns during development. *Hdac1* and *hdac3* (Class I) are highly expressed from 2–4 cells until 7 dpf, when visual function is matured. *Sirt7*, *hdac7* and *hdac11* (Class III, II and IV respectively) show higher expression at earlier stages, while *sirt2* and *hdac9b* (Class II) show increased gene expression after 6 hpf or at later developmental stages.

To begin to explore the importance of HDACs in the zebrafish eye, we profiled HDAC gene expression in eyes from 3, 4 and 5 dpf larvae (Yin et al. 2012). As shown in Fig. 61.2, *hdac1* and *hdac3* show similar decreasing expression from 3–5 dpf. In contrast expression of *hdac9b* significantly increased from 3–5 dpf. The differential expression of *hdac*s during the development of visual function indicates a temporal importance of HDAC expression during eye development. Other *hdac* genes did not exhibit any significant difference in gene expression from 3 to 5 dpf.

With the notable exception of *hdac1*, the role of most HDAC genes in the zebrafish eye is poorly understood. The absence of *hdac1* in the zebrafish retina results in increased cell proliferation, the optic stalk fails to terminally differentiate resulting in a reduced plexiform layer and number of retinal ganglion cells, photoreceptors are also absent (Stadler et al. 2005). *hdac1* is necessary for controlling

transcription of the key cell cycle regulators cyclin D1 and E2. *hdac1* appears to be required for the switch from proliferation to differentiation in the zebrafish retina mediated by the Wnt and Notch pathways (Yamaguchi et al. 2005).

## 61.6 Conclusion

Clinical and pre-clinical studies suggest that HDACi may be effective therapeutics in certain models of retinal degeneration. Zebrafish are an excellent model to gain further insight into the requirement of HDACs for eye development and function. Aditionally, zebrafish models of inherited blindness can be utilised to determine the efficacy and safety of HDACi in genetically diverse models of retinal degeneration and to understand the neuroprotective mechanisms of HDACi.

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