Role of HDACs in Retinal Degeneration

J Sancho-Pelluz[1,2], M Alavi[3,4], A Sahaboglu-Tekgoz[1], S Kustermann[1], T van Veen[1,4], FJ Romero[2], F Paquet-Durand[1], P Ekström[4]

1Division of Experimental Ophthalmology, Centre for Ophthalmology, Tübingen, GERMANY. 2Fundación Oftalmológica del Mediterráneo (FOM), Valencia, SPAIN. 3Molecular Genetics Laboratory, Institute for Ophthalmic Research, Centre for Ophthalmology, Tübingen, GERMANY. 4Dept. Ophthalmology, Clinical Sciences, Lund, University of Lund, SWEDEN.

contact: javier.sancho-pelluz@klinikum.uni-tuebingen.de

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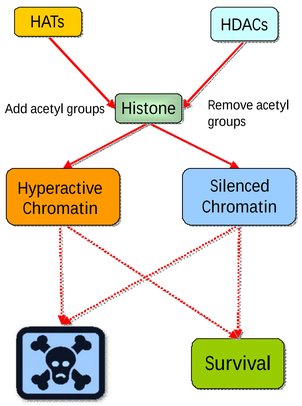
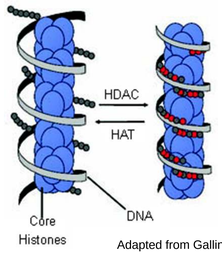
Introduction

Histones are components of the chromatin, and play a very important role in gene regulation. Histone acetylation is a posttranslational modification that leads to changes in chromatin structure and transcription. Histone acetyl transferases (HATs) add acetyl groups to the lysine residues, while histone deacetylases (HDACs) remove them (Kruszewski and Szumiel, 2005). Hyperacetylation may enhance expression of pro apoptotic transcription factors (apaf, caspase-3) (Wallace et al., 2006), as well as other factors critical for rod differentiation (Otx2, Nrl, Crx, Neurod 1) (Chen and Cepko, 2007). The aim of this study was to investigate the relevance of histone acetylation or deacetylation in rd photoreceptor survival.





Adapted from Gallinari et al., 2007

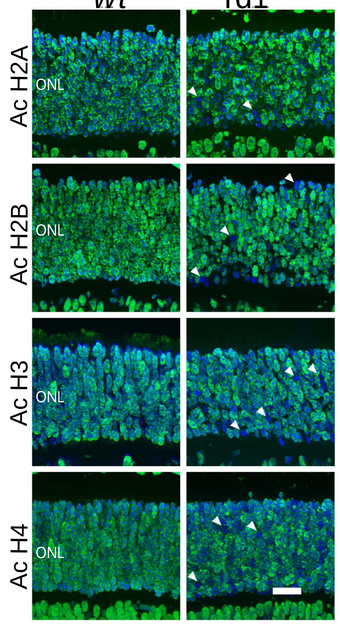
Results (I):Histone Acetylation and cell death in *wt* and rd1 retinae

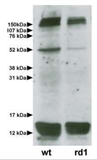
I.a.Immunofluorescence

At P11, immuno-fluorescence for different acetylated nucleosomal histone (Histone H2A, H2B, H3, and H4) lysine residues (green) on *wt* and rd1 retina reveals a number of rd1 photoreceptor nuclei that show very low levels of protein acetylation (arrowheads). In *wt*, the acetylation of lysine residues seems to be homogeneously distributed all along the ONL.

DAPI (blue) was used for nuclear counterstaining.

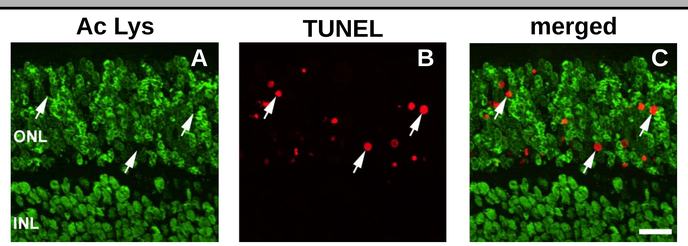
Bar: 20µm.





I.b. Western Blot

Lower protein acetylation in rd1 was confirmed at P11, using an antibody for acetylated lysine residues in western blotting. It revealed different bands at around 12-17 kDa that correspond to the histones. Less acetylation of the histones in the retina of the rd1 mouse, comparing with the *wt*, was observed. At 52 kDa another band, corresponding to tubulin, was also observed to be weaker in rd1 retina.

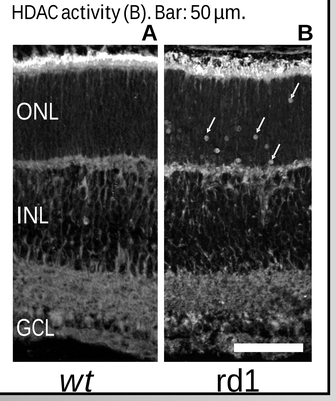


I.c.Acetylation and Cell Death

Low acetylation levels in PN11 rd1 photoreceptors are often associated with positive TUNEL reaction , suggesting that hypoacetylation may contribute to photoreceptor cell death. In a staining for acetylated lysine residues, a number of non-acetylated cells were noticed(gaps in the staining, arrows, A). With the TUNEL assay, the cells under degeneration are visible (arrows, B). The 2 pictures merged, showing us how the TUNEL positive cells are, in most of the cases non-acetylated cells, filling the “gaps” (arrows, C). Bar: 20µm.

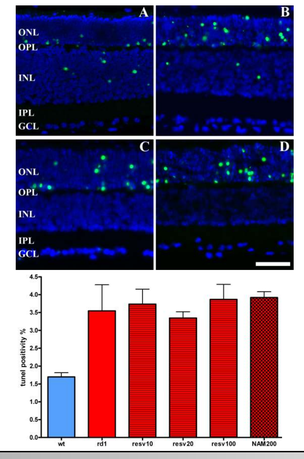
Results (II): HDAC activity in *wt* and rd1 retinae

Retinal sections from *wt* and rd1 mice have been incubated with 200 µM SirtII Substrate (Biomol). This assay was employed to resolve HDAC activity in individual retinal cell types. In *wt* tissue HDAC activity seems to be present predominantly in the photoreceptor segments, while all nuclear layers of the retina are essentially devoid of HDAC activity (A). In contrast to this, in the rd1 retina a subset of cell bodies in the ONL shows strong elevations of HDAC activity (B). Bar: 50 µm.



Results (III): HDAC alteration and Cell Death

Degenerating cells were measured with TUNEL assay in sections from retinal cultures of *wt* (A), rd1 untreated (B), rd1 treated with resveratrol (resv) at different concentrations (20 µM in C), and with nicotinamide (NAM) 200µM (D). Differences in the number of dying cells were determined comparing the healthy animal and rd1 (see bar diagram), but not significant differences where found when comparing the rd1 untreated animal with the treated ones. Bar: 50µm



Conclusions

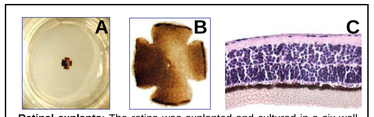
- The acetylation balance in the retina of rd1 mice seems to be altered.
- HDACs may be involved in the development and progression of photoreceptor degeneration in the rd1 mouse.
- The manipulation of HDACs class III (sirtuins) do not rescue the cells.

Future

- Delineate the precise role of HDACs in the degeneration process in the rd1 retina.
- Evaluate their potential for a treatment of photoreceptor cell death.

Methods

Immunofluorescence and Western Blot experiments were performed to study general acetylation in the photoreceptor cell layer of *wt* and rd1 retinae. Immunostainings for specific acetylated nucleosomal histones were also used. A newly adapted in situ activity assay was performed to study activity of HDACs in rd1 and *wt* retinae. Retinae from both groups of animals were cultured in an organotypic explant system and treated with specific inhibitors and activators HDACs class III (a.k.a. Sirtuins), . Cell death was assessed using TUNEL assay.



Retinal explants: The retina was explanted and cultured in a six-well plate from post natal day (P) 5 to P11(**A**) (Higuer magnification in **B**). 12 µm thick sections were prepare using a cryostat (**C**). Pictures by courtesy of Ekström.

Antibody	Dilution for IF	Dilution for WB	Company
Acetylated lysine	1:100	1:1000	Cell signaling, Danvers, MA, USA
Acetylated H2A	1:100	1:1000	Cell signaling
Acetylated H2B	1:100	1:1000	Cell signaling
Acetylated H3	1:100	1:1000	Cell signaling
Acetylated H4	1:100	1:1000	Cell signaling

Table 1: Primary antibodies used for Immunofluorescence (IF) and Western Blotting (WB) approaches.

Abbreviations

ONL: Outer Nuclear Layer; OPL: Outer Plexiform Layer; INL: Inner Nuclear Layer; IPL: Inner Plexiform Layer; GCL: Ganglion Cell Layer

Acknowledgments

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