Published in final edited form as:

Adv Exp Med Biol. 2018 ; 1074: 561–567. doi:10.1007/978-3-319-75402-4_68.

Role of Sirtuins in Retinal Function Under Basal Conditions

J. B. Lin[#],

Department of Ophthalmology & Visual Sciences, Washington University School of Medicine, St. Louis, MO, USA

Neuroscience Graduate Program, Division of Biology and Biomedical Sciences, Washington University School of Medicine, St. Louis, MO, USA

S. Kubota[#],

Department of Ophthalmology & Visual Sciences, Washington University School of Medicine, St. Louis, MO, USA

R. Mostoslavsky, and

Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA

R. S. Apte

Department of Ophthalmology & Visual Sciences, Washington University School of Medicine, St. Louis, MO, USA

Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO, USA

Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA e-mail: apte@vision.wustl.edu

[#] These authors contributed equally to this work.

Abstract

Sirtuins are NAD⁺-dependent enzymes that govern cellular homeostasis by regulating the acylation status of their diverse target proteins. We recently demonstrated that both rod and cone photoreceptors rely on NAMPT-mediated NAD⁺ biosynthesis to meet their energetic requirements. Moreover, we found that this NAD⁺-dependent retinal homeostasis relies, in part, on maintenance of optimal activity of the mitochondrial sirtuins and of SIRT3 in particular. Nonetheless, it is unknown whether other sirtuin family members also play important roles in retinal homeostasis. Our results suggest that SIRT1, SIRT2, SIRT4, and SIRT6 are dispensable for retinal survival at baseline, as individual deletion of each of these sirtuins does not cause retinal degeneration by fundus biomicroscopy or retinal dysfunction by ERG. These findings have significant implications and inform future studies investigating the mechanisms underlying the central role of NAD⁺ biosynthesis in retinal survival and function.

Keywords

Sirtuins; NAD⁺; Retinal degeneration; Neurodegeneration; Photoreceptors; Retina

68.1 Introduction

Sirtuins are NAD⁺-dependent enzymes that are critically important for maintaining cellular homeostasis, especially under conditions of metabolic and nutritional stress.

Mechanistically, sirtuins regulate cellular homeostasis by modifying the acylation status of their diverse target proteins. Since sirtuins require NAD⁺ as a cosubstrate, their function is exquisitely dependent on NAD⁺ availability (Imai et al. 2000; Verdin 2015). The sirtuin family includes numerous members, which localize to unique subcellular compartments: SIRT1 and SIRT2 are present in the nucleus and the cytoplasm (North and Verdin 2007; Tanno et al. 2007); SIRT6 is exclusively nuclear (Liszt et al. 2005); and SIRT3, SIRT4, and SIRT5 are present in the mitochondria (Schwer et al. 2002; Michishita et al. 2005). We recently demonstrated that NAMPT-mediated NAD⁺ biosynthesis is essential for photoreceptor survival and vision and that SIRT3 and SIRT5 play important roles in retinal homeostasis (Lin et al. 2016). However, whether the other mitochondrial sirtuin SIRT4 and the nuclear/cytoplasmic sirtuins, SIRT1, SIRT2, and SIRT6, play important roles in retinal function under baseline conditions has not been adequately explored. Therefore, we examined the effect of deleting these individual sirtuins on retinal survival and function.

68.2 Materials and Methods

68.2.1 Animals

All animal experiments were approved by the Animal Studies Committee and performed in accordance with the Washington University School of Medicine Animal Care and Use guidelines. We purchased floxed SIRT1 mice (*Sirt1^{F/F}*), *Sirt2^{-/-}* mice, and *Sirt4^{-/-}* mice, along with strain-matched controls, from Jackson Laboratory (Bar Harbor, ME). We received floxed SIRT6 mice (*Sirt6^{F/F}*) from Dr. Raul Mostoslavsky (Sebastian et al. 2012), rhodopsin-iCre75 transgenic mice from Dr. Ching-Kang Jason Chen (Li et al. 2005), and human red/green pigment-Cre (HRGP-Cre) transgenic mice from Dr. Yun Le (Le et al. 2004). We tested all mice by fundus biomicroscopy and electroretinography at roughly 2–3 months of age. Whenever possible, we compared mice to littermate controls. We confirmed that all mice were negative for the *rd8* mutation of the *Crb1* gene (Mattapallil et al. 2012) by PCR.

68.2.2 Assessment of Retinal Structure and Function

To assess retinal structure, we performed fundus biomicroscopy with the Micron III™ animal fundus camera (Phoenix Research Laboratories, Pleasanton, CA), as described previously (Lin et al. 2016). We examined multiple mice per genotype and present representative images. To assess retinal function, we performed electroretinography (ERG) with the UTAS-E3000 Visual Electrodiagnostic System running EM for Windows (LKC Technologies, Gaithersburg, MD), as described previously (Lin et al. 2016), testing at least three mice per genotype. For statistical analysis of the ERG data, we used GraphPad Prism version 5.0f (La Jolla, CA) to perform a mixed two-way ANOVA (within-subjects factor, flash intensity; between-subjects factor, genotype). We defined statistical significance as a p value <0.05.

68.3 Results

68.3.1 Deletion of *Sirt1*, *Sirt2*, *Sirt4*, or *Sirt6* Does Not Cause Retinal Degeneration

To determine whether deletion of individual sirtuins causes retinal degeneration, we assessed the fundus appearance of mice lacking various individual sirtuins, including rod photoreceptor-specific *SIRT1* conditional knockout (*Sirt1*^{rod/-rod}), cone photoreceptor-specific *SIRT1* conditional knockout (*Sirt1*^{cone/-cone}), *SIRT2* germline knockout (*Sirt2*^{-/-}), *SIRT4* germline knockout (*Sirt4*^{-/-}), rod photoreceptor-specific *SIRT6* conditional knockout (*Sirt6*^{rod/-rod}), and cone photoreceptor-specific *SIRT6* conditional knockout (*Sirt6*^{cone/-cone}) mice. We tested photoreceptor-specific conditional knockouts for *SIRT1* and *SIRT6* because *SIRT1* germline knockouts on our desired inbred background die shortly after birth (McBurney et al. 2003) and *SIRT6* germline knockouts have a severe neurodegenerative phenotype, dying around 4 weeks of age (Mostoslavsky et al. 2006). At 2–3 months of age, none of the mice we tested had any evidence of retinal degeneration from their fundus appearances (Fig. 68.1a–d). These findings suggest that *SIRT1*, *SIRT2*, *SIRT4*, and *SIRT6* are dispensable for retinal survival at baseline.

68.3.2 Deletion of *Sirt1*, *Sirt2*, *Sirt4*, or *Sirt6* Does Not Cause Retinal Dysfunction

To determine whether deletion of individual sirtuins causes baseline retinal dysfunction, we also tested these mice with ERG. Consistent with the normal retinal appearances on fundus biomicroscopy, *Sirt1*^{rod/-rod}, *Sirt1*^{cone/-cone}, *Sirt2*^{-/-}, *Sirt4*^{-/-}, *Sirt6*^{rod/-rod}, and *Sirt6*^{cone/-cone} mice all had normal retinal function at baseline at 2–3 months of age (Fig. 68.2a–d). Once again, these findings suggest that these sirtuins are not essential for retinal function under basal conditions.

68.4 Discussion

Sirtuins have been reported to play important functions in various mouse models of retinal disease (Shindler et al. 2007; Jaliffa et al. 2009; Chen et al. 2013; Zuo et al. 2013; Zeng and Yang 2015). However, we found that individual deletion of *Sirt1*, *Sirt2*, *Sirt4*, or *Sirt6* did not cause retinal degeneration as assessed by fundus biomicroscopy nor did it cause retinal dysfunction by ERG. These findings suggest that these sirtuins are individually dispensable under basal conditions. These findings are important, as we previously demonstrated that NAMPT-mediated NAD⁺ biosynthesis is essential for retinal function and that *SIRT3*/*SIRT5* may play important roles in NAD⁺-dependent retinal homeostasis under conditions of stress (Lin et al. 2016). These results expand on our previous findings and clarify the role of various sirtuin family members in retinal survival and function.

Further studies are necessary to determine whether these sirtuins become essential under conditions of stress, such as in light-induced degeneration or in other models of retinal dysfunction or retinal disease, since sirtuin-deficient mice tend not to show severe phenotypes until challenged by metabolic perturbations such as fasting (Hirschey et al. 2011). Moreover, our experiments in rod- and cone-specific conditional knockouts do not rule out the possibility that *SIRT1* and *SIRT6* play important roles in non-photoreceptor retinal cells. Indeed, *SIRT6* germline knockout mice exhibit retinal dysfunction on ERG by

3 weeks of age, which has been hypothesized to be related to an important role for SIRT6 in inner retinal cells, such as bipolar cells, Müller cells, or perhaps both (Silberman et al. 2014). Cumulatively, our results suggest that SIRT1, SIRT2, SIRT4, and SIRT6 are individually dispensable for retinal survival and function under basal conditions, although further studies are necessary to explore how they interact with one another and whether they become essential under disease conditions.

Acknowledgments

This work was supported by NIH Grants R01 EY019287 (R.S.A.) and P30 EY02687 (Vision Core Grant); the C.M. and M.A. Reeves Foundation (R.S.A.); Research to Prevent Blindness (R.S.A.); the Hope Center (R.S.A.); the Lacy Foundation (S.K.); the Schalak Family Gift Fund for Retinal Research (R.S.A.); the Jeffrey Fort Innovation Fund (R.S.A.); and the Robert Machemer Foundation (S.K.). Additional funding comes from an unrestricted grant to the Department of Ophthalmology and Visual Sciences of Washington University School of Medicine from Research to Prevent Blindness. J.B.L. was supported by the Washington University in St. Louis Medical Scientist Training Program (NIH Grant T32 GM007200), the Washington University in St. Louis Institute of Clinical and Translational Sciences (NIH Grants UL1 TR000448, TL1 TR000449), the Washington University Diabetic Cardiovascular Disease Center, the American Federation for Aging Research, and the VitreoRetinal Surgery Foundation.

References

- Chen J, Michan S, Juan AM et al. (2013) Neuronal sirtuin1 mediates retinal vascular regeneration in oxygen-induced ischemic retinopathy. *Angiogenesis* 16:985–992 [PubMed: 23912262]
- Hirschey MD, Shimazu T, Jing E et al. (2011) SIRT3 deficiency and mitochondrial protein hyperacetylation accelerate the development of the metabolic syndrome. *Mol Cell* 44:177–190 [PubMed: 21856199]
- Imai S, Armstrong CM, Kaeberlein M et al. (2000) Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 403:795–800 [PubMed: 10693811]
- Jaliffa C, Ameqrane I, Dansault A et al. (2009) Sirt1 involvement in rd10 mouse retinal degeneration. *Invest Ophthalmol Vis Sci* 50:3562–3572 [PubMed: 19407027]
- Le YZ, Ash JD, Al-Ubaidi MR et al. (2004) Targeted expression of Cre recombinase to cone photoreceptors in transgenic mice. *Mol Vis* 10:1011–1018 [PubMed: 15635292]
- Li S, Chen D, Sauve Y et al. (2005) Rhodopsin-iCre transgenic mouse line for Cre-mediated rod-specific gene targeting. *Genesis* 41:73–80 [PubMed: 15682388]
- Lin JB, Kubota S, Ban N et al. (2016) NAMPT-Mediated NAD(+) biosynthesis is essential for vision in mice. *Cell Rep* 17:69–85 [PubMed: 27681422]
- Liszt G, Ford E, Kurtev M et al. (2005) Mouse Sir2 homolog SIRT6 is a nuclear ADP-ribosyltransferase. *J Biol Chem* 280:21313–21320 [PubMed: 15795229]
- Mattapallil MJ, Wawrousek EF, Chan CC et al. (2012) The Rd8 mutation of the Crb1 gene is present in vendor lines of C57BL/6N mice and embryonic stem cells, and confounds ocular induced mutant phenotypes. *Invest Ophthalmol Vis Sci* 53:2921–2927 [PubMed: 22447858]
- McBurney MW, Yang X, Jardine K et al. (2003) The mammalian SIR2alpha protein has a role in embryogenesis and gametogenesis. *Mol Cell Biol* 23:38–54 [PubMed: 12482959]
- Michishita E, Park JY, Burneskis JM et al. (2005) Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. *Mol Biol Cell* 16:4623–4635 [PubMed: 16079181]
- Mostoslavsky R, Chua KF, Lombard DB et al. (2006) Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. *Cell* 124:315–329 [PubMed: 16439206]
- North BJ, Verdin E (2007) Interphase nucleo-cytoplasmic shuttling and localization of SIRT2 during mitosis. *PLoS One* 2:e784 [PubMed: 17726514]
- Schwer B, North BJ, Frye RA et al. (2002) The human silent information regulator (Sir)2 homologue hSIRT3 is a mitochondrial nicotinamide adenine dinucleotide-dependent deacetylase. *J Cell Biol* 158:647–657 [PubMed: 12186850]

- Sebastian C, Zwaans BM, Silberman DM et al. (2012) The histone deacetylase SIRT6 is a tumor suppressor that controls cancer metabolism. *Cell* 151:1185–1199 [PubMed: 23217706]
- Shindler KS, Ventura E, Rex TS et al. (2007) SIRT1 activation confers neuroprotection in experimental optic neuritis. *Invest Ophthalmol Vis Sci* 48:3602–3609 [PubMed: 17652729]
- Silberman DM, Ross K, Sande PH et al. (2014) SIRT6 is required for normal retinal function. *PLoS One* 9:e98831 [PubMed: 24896097]
- Tanno M, Sakamoto J, Miura T et al. (2007) Nucleocytoplasmic shuttling of the NAD⁺-dependent histone deacetylase SIRT1. *J Biol Chem* 282:6823–6832 [PubMed: 17197703]
- Verdin E (2015) NAD(+) in aging, metabolism, and neurodegeneration. *Science (New York, NY)* 350:1208–1213
- Zeng Y, Yang K (2015) Sirtuin 1 participates in the process of age-related retinal degeneration. *Biochem Biophys Res Commun* 468:167–172 [PubMed: 26522222]
- Zuo L, Khan RS, Lee V et al. (2013) SIRT1 promotes RGC survival and delays loss of function following optic nerve crush. *Invest Ophthalmol Vis Sci* 54:5097–5102 [PubMed: 23821198]

Fig. 68.1. *Sirt1*^{rod/rod} (a), *Sirt1*^{cone/cone} (a), *Sirt2*^{-/-} (b), *Sirt4*^{-/-} (c), *Sirt6*^{rod/rod} (d), and *Sirt6*^{cone/cone} (d) mice all have normal-appearing fundi on retinal imaging at 2–3 months of age

Fig. 68.2. *Sirt1*^{-rod/-rod} (a), *Sirt1*^{-cone/-cone} (a), *Sirt2*^{-/-} (b), *Sirt4*^{-/-} (c), *Sirt6*^{-rod/-rod} (d), and *Sirt6*^{-cone/-cone} (d) mice all have normal scotopic and photopic retinal function by ERG at 2–3 months of age (N.S. by two-way mixed ANOVA). Graphs depict mean ± S.E.M (a–d)