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Daily dynamics of autophagy gene expression in the rat retina during the development of retinopathy

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Motivation and Aim: Age-related macular degeneration (AMD) is a complex neurodegenerative progressive eye disease, resulting in severe loss of central vision in the aging population. AMD pathogenesis is not completely known, but strongly associate with autophagy disruption. Autophagy is a conserved cellular degradation pathway for the breakdown of cytoplasmic components: damaged proteins and organelles. Besides a housekeeping function, autophagy is crucial for response to the stress. Defects in the autophagy are linked to aging and disease pathology. Circadian rhythms are a hallmark of physiology, but how such daily rhythms organize cellular catabolism is poorly understood. The retina has an own circadian system in that all retinal cells have their own oscillators which integrated for the rhythmic regulation of retinal physiology and function [1]. In disease states, dysregulation of the retinal circadian rhythms might further exacerbate the development of retinopathy. The daily autophagy demands in the RPE require precise gene regulation for the digestion and recycling of intracellular and POS components in lysosomes in response to light and stress conditions. The time course of daily fluctuations in the levels of autophagy occurring in the photoreceptors and RPE has not been characterized, and the mechanisms underlying the dynamic regulation of autophagy in these cells remains unknown. The purpose of this study was to determine the daily pattern of autophagy genes expression in the retina of young and old Wistar and senescence-accelerated OXYS rats which spontaneously reproduce the major signs of AMD: dystrophic alterations of the RPE, thinning of the neuroretina, and impairment of choroidal microcirculation [2].

Methods and Algorithms: Retinal tissues from 3-month and 21-month-old OXYS rats developing signs of AMD and Wistar rats (n = 5) were collected at ZT1, ZT8 and ZT16 in Zeitgeber time units, where ZT0 represents lights on (8:00 am) and ZT12 represents lights off (8:00 pm). Levels of autophagy markers expression (*Atg5, Atg7, Becn1, Gabarapl1, Nbr1, Map1lc3b, p62,* and *Ulk1*) were evaluated by qPCR using 3 reference genes (*Actb, Gapdh, Hprt1*).

Results: Changes in the mRNA levels of three key autophagy genes (*Atg5, p62, Ulk1*) were determined in relation to the time of day. For the *Atg7* gene, the level of *Atg7* in the retina depended on the age of the animals according to ANOVA (p = 0.02): post-hoc analysis showed that at ZT1, *Atg7* levels were higher in the retina of old OXYS rats than in young rats (p = 0.0006). In terms of daily dynamics, *Atg7* levels were lower at ZT8 and ZT16 than at ZT1 (p = 0.002 and p = 0.009 respectively) in old OXYS rats, but not in Wistar rats. Moreover, at ZT1, the retinal level of *Atg7* was higher in OXYS rats than in Wistar rats (p = 0.04). For the *p62* gene, ANOVA showed that *p62* mRNA levels

depended on the age of the rats ($p = 0.005$), with a comparison of group means showing significant differences between ages only for OXYS rats: at the time points ZT1 and ZT16, old rats had higher levels of *p62* than at 3 months of age ($p = 0.04$ and $p = 0.03$, respectively). We also observed significant diurnal changes in *p62* gene expression – a decrease at ZT8 compared to ZT1 ($p = 0.003$), and an increase at ZT16 compared to ZT8 ($p = 0.02$). In the case of the *Ulk1* gene, the level of *Ulk1* depended on the interaction of the factors genotype*age*time ($p = 0.04$). When comparing group means, significant differences were found between strains at 21 months of age at ZT8 ($p = 0.03$), with higher expression of the *Ulk1* gene in Wistar rats compared to OXYS rats. Post-hoc analysis showed significant diurnal changes in *Ulk1* gene expression: the level of *Ulk1* in old OXYS rats at was lower at ZT8 than at ZT1 ($p = 0.006$), at ZT16 the gene expression was higher than at ZT8 ($p = 0.048$).

Conclusion: At 21 months of age, OXYS rats have altered diurnal dynamics of expression of three key autophagy genes (*Atg7*, *p62*, *Ulk1*) in the retina compared to both Wistar rats of the same age and 3-month-old OXYS rats. No time-of-day (ZT1, ZT8, ZT16) or age (3-month-old rats and 21-month-old rats) related changes in autophagy gene expression (*Atg5*, *Atg7*, *Becn1*, *Gabarapl1*, *p62*, *Nbr1*, *Ulk1*) were detected in the retina of Wistar rats. These data may suggest that autophagy dysregulation occurs in the retina of prematurely ageing OXYS rats, which may lead to the progression of retinopathy development. Our study emphasizes the importance of the autophagic pathway in the pathogenesis of AMD and supports the view that disruption of circadian rhythms may influence retinal ageing.

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