Experimental and Molecular Pathology 101 (2016) 124–132

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

The influence of changes in expression of redox-sensitive genes on the development of retinopathy in rats

M.L. Perepechaeva [a,*], N.G. Kolosova [a,b,c], N.A. Stefanova [b], A.Zh. Fursova [b], A.Y. Grishanova [a]

[a] Institute of Molecular Biology and Biophysics, Timakova Str. 2/12, Novosibirsk 630117, Russia
[b] Federal Research Center Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences, Lavrentiev Ave. 10, Novosibirsk 630090, Russia
[c] Novosibirsk State University, Pirogova Str. 2, Novosibirsk 630090, Russia

ARTICLE INFO

Article history:
Received 14 March 2016
and in revised form 7 June 2016
Accepted 19 July 2016
Available online 25 July 2016

Keywords:
Retinopathy
Age-related macular degeneration
OXYS rats
OXYSb rats
AhR
Nrf2

ABSTRACT

Age-related macular degeneration (AMD) is a complex multifactorial disease of the elderly, with unclear pathogenesis; AMD is the leading cause of blindness. One of the destructive processes in AMD is oxidative stress, which leads to an imbalance in the processes responsible for production and detoxification of reactive oxygen species. The aryl hydrocarbon receptor (AhR) signaling pathway can participate in the development of oxidative stress, but the main regulator of antioxidant defense is nuclear factor, erythroid derived 2 (Nrf2). AhR-dependent oxidative stress can be attenuated by activation of Nrf2, and defects in the Nrf2 signaling pathway can increase sensitivity of the cell to oxidative stress. Our aim was to determine the role of the pro-oxidant (AhR-dependent) and antioxidant (Nrf2-dependent) systems in the pathogenesis of AMD using rats of OXYS strain and of OXYSb substrain with signs of AMD-like retinopathy of varying severity. We compared the retinal levels of mRNA expression of Nrf2- and AhR-dependent redox-sensitive systems between 1-, 3-, and 12- month-old senescence-accelerated OXYS rats (have been shown to be a valid experimental model of AMD) and the rat substrain OXYSb, which shows low morbidity of AMD. We uncovered interstrain differences in the expression of *Nrf2* and *Nrf2*-dependent genes (glutathione S-reductase [*Gsr*] and heme oxygenase 1 [*Hmox1*]), in the expression of AhR-dependent genes (cytochrome P450 1A2 [*Cyp1a2*] and cytochrome P450 1B1 [*Cyp1b1*]), and in the NADPH-quinone oxidoreductase (*Nqo1*) expression, which is controlled by both AhR and Nrf2. Binding of AhR and Nrf2 proteins to the regulatory regions of *AhR* and *Nrf2* genes, respectively, was detected by chromatin immunoprecipitation in the retina of 1-, 3-, and 12-month-old OXYS, OXYSb, and Wistar (control) rats. We compared the strength of DNA-protein interactions of AhR and Nrf2 with regulatory sequences and found that the level of autoupregulation of the *AhR* gene was higher in the retina of 1-month-old OXYSb rats in comparison with OXYS rats. An imbalance between pro-oxidant (AhR-dependent) and antioxidant (Nrf2-dependent) systems may play a crucial role in the onset and/or progression of AMD.

1. Introduction

Age-related macular degeneration (AMD) is a cause of blindness in tens of millions of people worldwide (Tuo et al., 2012). AMD is a complex multifactorial neurodegenerative disease, whose etiology involves genetic, environmental, and demographic factors (Ratnapriya and Chew, 2013). The pathogenesis of AMD is believed to be based on aging-specific structural functional alterations in the retina, which do not always lead to the development of the disease. One of the important components of AMD pathogenesis is believed to be the development of oxidative stress, when overproduction of reactive oxygen species leads to atrophy of the retinal pigment epithelium (RPE) (Lambros and Plafker, 2016; Nita and Grzybowski, 2016). Retinal tissue actively consumes oxygen, and at the same time, its surroundings are conducive to formation of reactive oxygen species and consequently to oxidative damage (Jarrett and Boulton, 2012). Normally, antioxidant systems can manage the workload, but when an imbalance appears for some reason, a pathological process develops. Oxidative stress in AMD can be described as a result of excessive production of reactive oxygen species (Totan et al., 2009; Venza et al., 2012), disturbances of the antioxidant system (Jarrett and Boulton, 2012; Yildirim et al., 2011), mitochondrial dysfunction (Feher et al., 2006; Jarrett et al., 2008), or a combination of the above factors. In some studies, it has been shown

Abbreviations: AHR, aryl hydrocarbon receptor; Aldh3a1, aldehyde dehydrogenase 3A1; AMD, age-related macular degeneration; BHQ, black-hole quencher; CYP1A1, cytochrome P450 1A1; CYP1A2, cytochrome P450 1A2; CYP1B1, cytochrome P450 1B1; FAM, 6-carboxyfluorescein; Gsr, glutathione-S-reductase; Gsta1, glutathione S-transferase A1; Hmox1, heme oxygenase 1; Nqo1, NADPH-quinone oxidoreductase; Nrf2, nuclear factor erythroid derived 2; RPE, retinal pigment epithelium; Txnrd1, thioredoxin reductase 1; UDP, uridine diphosphate; Ugt1a6, UDP-glucuronosyltransferase 1A6; Ugt1a9, UDP-glucuronosyltransferase 1A9; VEGF-A, vascular endothelial growth factor A.

* Corresponding author.
E-mail addresses: perepech@niimbb.ru (M.L. Perepechaeva), kolosova@bionet.nsc.ru (N.G. Kolosova), stefanovan@bionet.nsc.ru (N.A. Stefanova), anzhellafursova@yandex.ru (A.Z. Fursova), agrish@niimbb.ru (A.Y. Grishanova).

http://dx.doi.org/10.1016/j.yexmp.2016.07.008
0014-4800/

that the imbalance of parameters of oxidative and antioxidant systems in patients with AMD can play a role in the pathogenesis of this disease (Totan et al., 2009; Venza et al., 2012; Yildirim et al., 2011).

Molecular genetic mechanisms underlying the transition from age-related changes to pathological processes are not known. Weakening of cellular functions during aging is linked to changes in the expression of numerous genes. Transcriptomic studies can help to identify those genes and to determine the metabolic pathways where changes in activity levels lead to age-related alterations and to the development of age-related diseases.

There is a growing body of evidence of genes that can predispose a person to AMD or drive the relevant pathophysiological processes. One such gene is nuclear factor, erythroid derived 2 (*Nrf2*), a redox-dependent transcription factor and the main activator of molecular antioxidant pathways, in particular, those involving the enzymes heme oxygenase 1 (Hmox1), thioredoxin reductase 1 (Txnrd1), and glutathione-S-reductase (Gsr) (Kato et al., 2010). The involvement of *Nrf2* in retinal diseases was shown indirectly (Zhao et al., 2011); namely, those authors demonstrated development of age-related retinopathy in *Nrf2*-deficient mice: the pathology was similar in its manifestations to human AMD, and recently it was demonstrated that human AMD can be associated with a polymorphism of the *Nrf2* gene (Sliwinski et al., 2013).

Because of some evidence about the association of AMD with a polymorphism of Aryl hydrocarbon receptor (AhR) (Esfandiary et al., 2005), *AhR* was suggested to be a possible gene associated with predisposition to AMD. AhR is a ligand-dependent transcription factor that controls expression of a number of enzymes metabolizing xenobiotics, including enzymes of phase I of xenobiotic metabolism: cytochromes P450 of subfamily 1A (CYP1A1, CYP1A2, and CYP1B1); activity of these enzymes leads to formation of reactive oxygen species (Ma, 2001; Whitlock, 1999).

On the other hand, glutathione S-transferase A1 (Gsta1), NAD(P)H:quinone oxidoreductase 1 (Nqo1), UDP-glucuronosyl transferase 1A6 (Ugt1A6), UDP-glucuronosyl transferase 1A9 (Ugt1A9), and several isoforms of UGT and GST are controlled by both AhR and Nrf2 (Yeager et al., 2009). There are few empirical studies on the gene expression of redox-sensitive systems in the retina. One such report (Zacks et al., 2006) showed that oxidative-stress–dependent retinal detachment is mediated by AhR; the role of AhR in the pathogenesis of AMD is also discussed by other researchers (Dwyer et al., 2011).

Research into the pathogenic processes of AMD is often hampered by the difficulties with experiments on humans and by the limitations of animal models. We previously showed that senescence-accelerated OXYS rats are a suitable model for studies on the pathogenesis and possible therapeutic targets of AMD (Korbolina et al., 2012; Kozhevnikova et al., 2013; Markovets et al., 2011). The OXYS rat strain was developed at the Institute of Cytology and Genetics from Wistar stock by selection on the basis of susceptibility to cataractogenic effects of a galactose-rich diet and by inbreeding of highly susceptible rats, as described earlier (Rumyantseva et al., 2008; Solov'eva et al., 1975). Today we have the 107th generation of OXYS rats with spontaneously developing cataract and the accelerated-senescence phenotype inherited in a linked manner. In addition to cataract, this phenotype includes AMD-like retinopathy, high blood pressure, osteoporosis, and accelerated brain aging (Kozhevnikova et al., 2013). These signs are strongly associated with aging and are suggestive of a common underlying process. Retinopathy clinically similar to the initial stages of AMD appears in 100% of OXYS rats already by the age of 3–4 months, against the background of a decline in the number of RPE cells and alterations in choroidal microcirculation. Significant pathological changes of the RPE as well as signs of advanced stages of retinopathy are observed in OXYS rats older than 12 months. These aberrations manifest themselves as excessive accumulation of lipofuscin in the regions of RPE adjacent to the rod cells (in whirling extensions of the basement membrane into the cytoplasm). Eventually, primary cellular degenerative changes develop in the RPE cells, thus leading to choriocapillaris atrophy and a complete loss of photoreceptor cells in the OXYS retina by the age of 24 months (Korbolina et al., 2012; Kozhevnikova et al., 2013; Markovets et al., 2011).

We showed recently that in the retina of OXYS rats, changes in gene expression within the AhR- and Nrf2-dependent signaling pathways—in comparison with Wistar rats (no development of retinopathy)—can contribute to the development of an AMD-like pathology (Perepechaeva et al., 2014). Nevertheless, it is not clear whether the imbalance between antioxidant (Nrf2-dependent) and pro-oxidant (AhR-dependent) systems is necessary for the development of AMD or is merely a risk factor. A useful tool for elucidation of the AMD pathogenesis, in our opinion, can be the substrain OXYSb, which we generated from the original OXYS rats (of the 58th generation); this substrain differs from the parental strain by normal blood pressure and milder retinopathy.

Aside from the expression of AhR-dependent and Nrf2-dependent genes, it is also important to analyze regulation of the transcription factors AhR and Nrf2 themselves, namely, the regulation that involves binding of the AhR and Nrf2 proteins to the regulatory regions of the genes *AhR* and *Nrf2*, respectively. Upstream of the transcription start site of the *AhR* gene, there is a xenobiotic responsive element (XRE)-containing region, which serves as a regulatory sequence responsible for AhR autoregulation (Singhal et al., 2008). Nrf2 binds directly to an antioxidant-responsive element (ARE) in its own promoter and autoregulates its own expression (Bryan et al., 2013). In the present work, we assessed the age-related changes in AhR and Nrf2 autoregulation, mRNA levels of *AhR* and of the AhR-regulated "gene battery" as well as the expression levels of *Nrf2* and of the Nrf2-regulated gene battery. We also evaluated the expression of genes regulated by both AhR and Nrf2 in the retina of OXYS rats (high morbidity of AMD) and OXYSb rats (low morbidity of AMD).

2. Materials and methods

2.1. The ethics statement

The animal experiments in this paper comply with the Principles of Laboratory Animal Care (NIH publication No. 85–23, revised 1985), the OPRR Public Health Service Policy on the Humane Care and Use of Laboratory Animals (revised 1986), and the U.S. Animal Welfare Act, as amended, as well as the relevant Russian laws and regulations.

2.2. Animals

Male senescence-accelerated OXYS rats ($n = 8$ to 12), age-matched male OXYSb rats ($n = 8$ to 12), and (for a chromatin immunoprecipitation [ChIP] assay) Wistar rats ($n = 3$) were obtained from the Breeding Experimental Animal Laboratory of the Institute of Cytology and Genetics, the Siberian Branch of the Russian Academy of Sciences (Novosibirsk, Russia). At age four weeks, the pups were weaned, housed in groups of five animals per cage ($57 \times 36 \times 20$ cm), and kept under standard laboratory conditions (22 °C ± 2 °C, 60% relative humidity, and 12 h/12 h light/dark cycle; lights on at 9 a.m.). The animals were provided with standard rodent feed (PK-120-1, Laboratorsnab, Ltd., Russia) and water *ad libitum*. Rats between 1 and 12 months of age were used in the experiments, as described below.

Ophthalmoscopic examination of rats' eyes was carried out using a "Heine BETA 200 TL" Direct Ophthalmoscope (Heine, Germany) after dilatation with 1% tropicamide. A Kowa Genesis-D fundus camera (Japan) was used as a hand-held digital camera for selective photography. Assessment of stages of retinopathy was conducted according to the grade protocol from the Age-Related Eye Disease Study (AREDS; http://eyephoto.ophth.wisc.edu or http://www.centreforeyehealth.com.au/uploads/62003/ufiles/downloads/guidelines/guideline5_2012_amd_web.pdf). Severity of retinopathy was estimated as follows: