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Glutathione S-Transferase Pi Isoform (GSTP1) Expression in Murine Retina Increases with Developmental Maturity

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Abstract

Background and Aims—Glutathione S-transferase pi isoform (GSTP1) is an intracellular detoxification enzyme that catalyzes reduction of chemically reactive electrophiles and is a zeaxanthin-binding protein in the human macula. We have previously demonstrated that GSTP1 levels are decreased in human age-related macular degeneration (AMD) retina compared to normal controls [1]. We also showed that GSTP1 levels parallel survival of human retinal pigment epithelial (RPE) cells exposed to UV light, and GSTP1 over-expression protects them against UV light damage [2]. In the present work, we determined the developmental time course of GSTP1 expression in murine retina and in response to light challenge.

Methods—Eyes from BALB/c mice at post-natal day 20, 1 month, and 2 months of age were prepared for retinal protein extraction and cryo sectioning, and GSTP1 levels in the retina were analyzed by Western blot and immunohistochemistry (IHC). Another group of BALB/c mice with the same age ranges was exposed to 1000 lux of white fluorescent light for 24 hours, and their retinas were analyzed for GSTP1 expression by Western blot and IHC in a similar manner.

Results—GSTP1 levels in the murine retina increased in ascending order from post-natal day 20, 1 month, and 2 months of age. Moreover, GSTP1 expression in murine retina at post-natal day 20, 1 month, and 2 months of age increased in response to brief light exposure compared to age-matched controls under normal condition.

Conclusions—GSTP1 expression in retina increases with developmental age in mice and accompanies murine retinal maturation. Brief exposure to light induces GSTP1 expression in the murine retina across various developmental ages. GSTP1 induction may be a protective response to light-induced oxidative damage in the murine retina.

Keywords

Glutathione S-transferase pi (GSTP1); retinal pigment epithelium (RPE); light toxicity; oxidative stress; retinal development; retinal degeneration; age-related macular degeneration (AMD)

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4.1 Introduction

4.1.1 GSTP1 and Oxidative Stress

Glutathione S-transferases (GSTs) are a family of intracellular detoxification enzymes that catalyze the reduction of electrophiles by conjugating them to glutathione. The human GSTs are classified into at least four families: alpha, mu, pi, and theta [3]. Different isomers exist for each of the four classes of GSTs, but only one isoform of the pi-class GST (GSTP1) is known to be expressed in human tissues. GSTP1 has been identified as a zeaxanthin-binding protein and found to be localized in the retina [4]. GSTP1 has been shown to play a role in oxidative damage in cancer [5–9]. GSTP1 also has been shown to protect against endothelial dysfunction induced by exposure to tobacco smoke in mice [10]. Overall, it is speculated that *GSTP1* is induced in order to scavenge toxic electrophiles, including reactive oxygen species. Thus, if GSTP1 expression is down-regulated, the cells become susceptible to oxidative damage leading to diseased states, such as age-related chronic degenerative disorders.

4.1.2 GSTP1 and Maturation

Not much is known about the association between GSTP1 and developmental maturation and aging. GSTP1 and GSTA3 proteins have been shown to increase in rat cochlea during early development [11]. GSTP1 and GSTA4 expression increased with age in rat cerebral cortex [12]. Intracellular translocation of GST-pi, a marker for mature oligodendrocytes in adult mammalian brain, from the nucleus to the cytosol occurs during oligodendrocyte differentiation in adult rat cerebral cortex [13].

4.1.3 Light Toxicity

High-energy photons can create free radicals which are damaging to DNA and cellular organelles such as mitochondria. It has been suggested that ultraviolet (UV) radiation may cause retinal damage and may contribute to the development of AMD [14]. Phototoxic damage also has been demonstrated in cultured human RPE cells [15, 16]. Animal studies have shown that excessive exposure to visible or UV light induced retinal damages to photoreceptors and RPE [17–19].

The retina is particularly susceptible to oxidative stress because of its high consumption of oxygen, high proportion of polyunsaturated fatty acids (PUFAs), and exposure to visible light [20, 21]. GSTP1's localization in the macula as a zeaxanthin-binding protein suggests that GSTP1 plays an important role in modulating the levels of antioxidants in the macula. We have previously demonstrated that GSTP1 levels are decreased in human AMD retina compared to normal controls. We also showed that GSTP1 levels parallel survival of human RPE cells exposed to UV light, and GSTP1 over-expression protects them against UV light damage. In the present work, we determined the developmental time course of GSTP1 expression in murine retina and in response to light challenge.

4.2 Materials and Methods

4.2.1 Experiment with Animals

All animal experiments were in accordance with the guidelines of the Declaration of Helsinki and the Association for Research in Vision and Ophthalmology, as approved by the University of Miami Institutional Animal Care and Use Committee.

4.2.2 Light Exposure

BALB/c mice at post-natal day 20, 1 month, and 2 months of age were exposed to 1000 lux of white fluorescent light for 24 hours. The age-matched control group of BALB/c mice was kept under normal condition. The eyes were enucleated and prepared for retinal protein extraction and for cryo sectioning.

4.2.3 Immunohistochemistry

The enucleated mouse eyes were embedded whole in O.C.T. (Tissue Tek), frozen at -80°C , cryo-sectioned, and stored at -20°C . Retinal sections were fixed with 4% paraformaldehyde and processed using standard protocol for IHC by probing with polyclonal antibodies (Abcam, Inc.) against murine GST3/GST pi protein (murine homolog of GSTP1), followed by secondary antibodies coupled to Alexa 488 (Invitrogen) showing green fluorescence. DAPI was used to stain nuclei (blue). The immunostaining was detected using a confocal Leica TSP microscope.

4.3.3 Western Blot Analysis

Protein extracts from mouse retinas were subject to Western blot analysis. The proteins were separated on 4–20% SDS-PAGE (Invitrogen), transferred onto a polyvinylidene fluoride (PVDF) membrane, blocked with 5% BSA in 0.2% TBST, and subsequently probed with polyclonal antibodies against murine GST3/GST pi protein (Abcam, Inc.), followed by secondary antibodies (Santa Cruz Biotechnology, Inc). For control, the membrane was also probed with polyclonal antibodies against murine GAPDH protein (Cell Signalling, Inc.), followed by secondary antibodies (Invitrogen). The Western blots were developed by electrochemiluminescence (ECL) (Pierce Biotechnology) and exposed to films.

4.3 Results

4.3.1 GSTP1 expression in retina accompanied murine retinal maturation

To determine the murine retinal GSTP1 expression at baseline in the early developmental spectrum, the retinas from BALB/c mice at post-natal day 20 (P20), 1 month (1mo), and 2 months (2mo) of age kept under normal light-dark cycle condition were assessed by IHC analysis. GSTP1 was detected in all layers of murine retina as early as post-natal day 20, and the intensity of GSTP1 expression increased correspondingly with retinal maturation as the age increased from P20 to 1 month to 2 months of age (Figure 4.1A. (–) **Light**). GSTP1 levels in retina from mice at P20, 1 month, and 2 months of age also increased in response to light exposure when compared to baseline (Figure 4.1B. (+) **Light**). The increase in GSTP1 expression was observed in all retinal layers and slightly more so in the RPE layer.

4.3.2 GSTP1 levels in murine retina increased with developmental age and with light exposure

Retinal GSTP1 levels from BALB/c mice at post-natal day 20 (P20), 1 month (1mo), and 2 months (2mo) of age kept under normal light-dark cycle condition (Figure 4.2A, B. (–) **Light**) were compared to those exposed to light for 24 hours (Figure 4.2C, D. (+) **Light**) by Western blot analysis, and the relative GSTP1 levels were quantified by optical density using the Image J software. GSTP1 levels in murine retina increased with developmental age from P20 to 1 month to 2 months. In addition, at each of these developmental stages, the retinal GSTP1 levels also were induced by exposure to light, some by nearly two folds.

4.4 Discussion

GSTP1 is an intracellular detoxification enzyme that catalyzes reduction of chemically reactive electrophiles and is a zeaxanthin-binding protein in the human macula. We have previously demonstrated that GSTP1 levels are decreased in human AMD retina compared to normal controls. We also showed that GSTP1 levels parallel survival of human RPE cells exposed to UV light, and GSTP1 over-expression protects them against UV light damage. In the present work, we determined the developmental time course of GSTP1 expression in murine retina and in response to light challenge.

In this study, we used BALB/c mice at ages P20, 1 month, and 2 months that represent roughly from infancy to sexual maturation in the early spectrum of development. We found that GSTP1 expression was present in murine retina as early as age P20. Our data demonstrated that GSTP1 expression in retina increased with developmental age in mice and accompanied murine retinal maturation. This suggests that GSTP1 is expressed in retina as early as P20 in murine development and increases in expression levels with retinal maturation as the mouse reaches sexual maturity. This also suggests that GSTP1 may play a role in normal development in the murine retina. We also showed that brief exposure to light induced GSTP1 expression in the murine retina across various developmental ages from P20 to 2 months. This suggests that GSTP1 induction may be a protective response to light-induced oxidative damage in the murine retina in normal development. It is tantalizing to speculate that disturbance in GSTP1 expression at baseline or failure to induce GSTP1 in response to oxidative stress may render the retina susceptible to damages leading to retinal degenerative disorders.

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References

1. Joshi PM, Franko M, Dubovy S, Bhattacharya SK, Lee W-H. Decreased expression of GSTP1 in the macula leads to AMD pathogenesis. Invest Ophthalmol Vis Sci. 2009 E-Abstract.

2. Joshi PM, Dubovy S, Bhattacharya SK, Lee W-H. Glutathione S-Transferase Pi isoform (GSTP1) expression plays a role in the health of retinal pigment epithelium (RPE). *Invest Ophthalmol Vis Sci*. 2010 E-Abstract.
3. Mannervik B, Awasthi YC, Board PG, Hayes JD, Di Ilio C, Ketterer B, Listowsky I, Morgenstern R, Muramatsu M, Pearson WR, Pickett CB, Sato K, Widersten M, Wolf CR. Nomenclature for human glutathione transferases. *Biochem J*. 2007; 282(Pt 1):305–306. [PubMed: 1540145]
4. Bhosale P, Larson AJ, Frederick JM, Southwick K, Thulin CD, Bernstein PS. Identification and characterization of a Pi isoform of glutathione S-transferase (GSTP1) as a zeaxanthin-binding protein in the macula of the human eye. *J Biol Chem*. 2004; 279(47):49447–49454. [PubMed: 15355982]
5. Ritchie KJ, Henderson CJ, Wang XJ, Vassieva O, Carrie D, Farmer PB, Gaskell M, Park K, Wolf CR. Glutathione transferase pi plays a critical role in the development of lung carcinogenesis following exposure to tobacco-related carcinogens and urethane. *Cancer Res*. 2007; 67(19):9248–9257. [PubMed: 17909032]
6. Huang J, Tan PH, Tan BK, Bay BH. GST-pi expression correlates with oxidative stress and apoptosis in breast cancer. *Oncol Rep*. 2004; 12(4):921–925. [PubMed: 15375523]
7. Matsui A, Ikeda T, Enomoto K, Hosoda K, Nakashima H, Omae K, Watanabe M, Hibi T, Kitajima M. Increased formation of oxidative DNA damage, 8-hydroxy-2'-deoxyguanosine, in human breast cancer tissue and its relationship to GSTP1 and COMT genotypes. *Cancer Lett*. 2000; 151(1):87–95. [PubMed: 10766427]
8. Fryer AA, Ramsay HM, Lovatt TJ, Jones PW, Hawley CM, Nicol DL, Strange RC, Harden PN. Polymorphisms in glutathione S-transferases and non-melanoma skin cancer risk in Australian renal transplant recipients. *Carcinogenesis*. 2005; 26(1):185–191. [PubMed: 15459020]
9. Lee WH, Morton RA, Epstein JI, Brooks JD, Campbell PA, Bova GS, Hsieh WS, Isaacs WB, Nelson WG. Cytidine methylation of regulatory sequences near the pi-class glutathione S-transferase gene accompanies human prostatic carcinogenesis. *Proc Natl Acad Sci USA*. 1994; 91(24):11733–11737. [PubMed: 7972132]
10. Conklin DJ, Haberzettl P, Prough RA, Bhatnagar A. Glutathione-S-transferase P protects against endothelial dysfunction induced by exposure to tobacco smoke. *Am J Physiol Heart Circ Physiol*. 2009; 296:1586–1597.
11. Whitton DS, Wright LS, Nelson SA, Szakaly R, Siegel FL. Maturation of cochlear glutathione-S-transferases correlates with the end of the sensitive period for ototoxicity. *Hear Res*. 1999; 137:43–50. [PubMed: 10545632]
12. Martinez-Lara E, Siles E, Hernandez R, Canuelo AR, del Moral ML, Jimenez A, Blanco S, Lopez-Ramos JC, Esteban FJ, Pedrosa JA, Peinado MA. Glutathione S-transferase isoenzymatic response to aging in rat cerebral cortex and cerebellum. *Neurobiol Aging*. 2003; 24:501–509. [PubMed: 12600725]
13. Tamura Y, Kataoka Y, Cui Y, Takamori Y, Watanabe Y, Yamada H. Intracellular translocation of glutathione S-transferase pi during oligodendrocyte differentiation in adult rat cerebral cortex in vivo. *Neuroscience*. 2007; 148:535–540. [PubMed: 17681700]
14. Braustein RE, Sparrow JR. A blue-blocking intraocular lens should be used in cataract surgery. *Arch Ophthalmol*. 2005; 123:547–549. [PubMed: 15824231]
15. Gao X, Talalay P. Induction of phase 2 genes by sulforaphane protects retinal pigment epithelial cells against photooxidative damage. *Proc Natl Acad Sci USA*. 2004; 101(28):10446–10451. [PubMed: 15229324]
16. Youn H-Y, Chou BR, Cullen AP, Sivak JG. Effects of 400 nm, 420 nm, and 435. 8 nm radiations on cultured human retinal pigment epithelial cells. *J Photochem Photobiol B*. 2009; 95:64–70. [PubMed: 19201202]
17. Organisciak DT, Darrow RM, Barsalou L, Kutty RK, Wiggert B. Susceptibility to retinal light damage in transgenic rats with rhodopsin mutations. *Invest Ophthalmol Vis Sci*. 2003; 44(2):486–492. [PubMed: 12556372]
18. Grimm C, Wenzel A, Hafezi F, Yu S, Redmond TM, Remé CE. Protection of Rpe65-deficient mice identifies rhodopsin as a mediator of light-induced retinal degeneration. *Nat Genet*. 2000; 25(1):63–6. [PubMed: 10802658]

19. Rattner A, Toulabi L, Williams J, Yu H, Nathans J. The genomic response of the retinal pigment epithelium to light damage and retinal detachment. *J Neurosci*. 2008; 28(39):9880–9. [PubMed: 18815272]
20. Bazan NG. The metabolism of omega-3 polyunsaturated fatty acids in the eye: the possible role of docosahexaenoic acid and docosanoids in retinal physiology and ocular pathology. *Prog Clin Biol Res*. 1989; 312:95–112. [PubMed: 2529559]
21. Dargel R. Lipid peroxidation--a common pathogenetic mechanism? *Exp Toxicol Pathol*. 1992; 44(4):169–181. [PubMed: 1392519]

Figure 4.1. GSTP1 in murine retina increased with retinal maturation and with light exposure by IHC

Representative images of IHC analysis of GSTP1 in the retina from BALB/c mice at post-natal day 20 (P20), 1 month (1mo), and 2 months (2mo) of age kept under normal light-dark cycle condition (**Figure 4.1A. (-) Light**), compared to those exposed to light for 24 hours (**Figure 4.1B. (+) Light**). Merged image (Panel A) and separate images showed GSTP1 staining as green fluorescence (Panel C) and DAPI staining the nuclei blue (Panel B.) (OD = Right eye).

Figure 4.2. GSTP1 in murine retina increased with developmental age and with light exposure by Western blot analysis

Retinal GSTP1 levels from BALB/c mice at post-natal day 20 (P20), 1 month (1mo), and 2 months (2mo) of age kept under normal light-dark cycle condition (**Figure 4.2A, B. (-) Light**) were compared to those exposed to light for 24 hours (**Figure 4.2C, D. (+) Light**). The optical density of each band was measured using the Image J software, and relative GSTP1 levels were quantified (**B, D**). (OD = Right eye, OS = Left eye).