Experimental Eye Research 152 (2016) 1–9

Contents lists available at ScienceDirect

Experimental Eye Research

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

Research article

Abnormal pigment epithelium-derived factor processing in progressive myopia

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ARTICLE INFO

Article history:
Received 28 February 2016
Received in revised form
28 July 2016
Accepted in revised form 29 August 2016
Available online 30 August 2016
This paper is dedicated to the memory of Dr. Irina A. Kostanian.

Keywords:
Amyloid
Myopia
Pigment epithelium-derived factor
Proteolytic degradation
Tenon's capsule

ABSTRACT

Pigment Epithelium-Derived Factor (PEDF) is a secreted glycoprotein belonging to the family of non-inhibitory serpins. It is known, that in cases of complicated myopia, the content of PEDF in aqueous humor of the anterior chamber is significantly reduced. Here we examined a bulk of Tenon's capsule samples obtained from various groups of myopes, to examine PEDF processing in progressive myopia. We have analyzed the distribution of full length PEDF50 and its truncated form PEDF45 in the soluble and insoluble fractions extracted from Tenon's capsule of myopic and control (non-myopic) patients using SDS-polyacrylamide gel electrophoresis, as well as monitored the proteolytic degradation of PEDF *ex vivo* by enzyme-linked immunosorbent assay. These results were complemented by PEDF mRNA analysis in correspondent tissues by using qPCR and immunohistochemistry analysis of PEDF distribution in normal and myopic specimens. We found that in the Tenon's capsule of patients suffering from a high myopia the level of "soluble" 45 kDa PEDF reduced by 2-fold, while the content of "insoluble" 50 kDa form of PEDF was increased by 4-fold compared to controls. Excessive amount of PEDF50 in myopic specimens have been shown to correlate with the abrogated PEDF processing rather than with an increase of its expression. Moreover, immunohistochemical staining of the myopic Tenon's capsule tissue sections revealed the halo of deposited PEDF50 in the fibroblast extracellular space. These findings suggest that in myopia limited proteolysis of PEDF is altered or abrogated. Accumulation of full-length PEDF insoluble aggregates in the fibroblast intercellular space may affect cell survival and consequently causes the destructive changes in the extracellular matrix of the eye connective tissues. As a result, the abrogation of full-length PEDF normal processing can be an important mechanism leading to biomechanical destabilization of the scleral capsule and myopia progression.

1. Introduction

Myopia is one of the most common eye diseases, which develops and progresses mostly in childhood and adolescence, and often leads to permanent loss of vision. The progression of myopia is associated with damage to the sclera – connective-tissue eye capsule, leading to significant alterations of its morphological structure, biochemical indicators and support function (Avetisov, 1999; Curtin, 1985; Iomdina, 2005). The development of disease is accompanied by degradation of collagen fibers of the extracellular matrix (McBrien et al., 2001), altered expression and activation of a range of proteins such as matrix metalloproteinases

Abbreviations: ELISA, enzyme-linked immunosorbent assay; PEDF, Pigment Epithelium-Derived Factor; PRD, peripheral retinal degeneration; TC, Tenon's capsule; WB, Western blotting.

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http://dx.doi.org/10.1016/j.exer.2016.08.017
0014-4835/

(Guggenheim and McBrien, 1996; Chen et al., 2014; Notari et al., 2005) and PEDF (Ogata et al., 2005; Tong et al., 2006).

PEDF is a secreted monomeric glycoprotein with a molecular weight of 50.1 kDa. It belongs to the family of non-inhibitory serpins and performs a wide range of functions, including differentiating, neurotrophic and anti-angiogenic activities (reviewed in Becerra, 1997; Jablonski et al., 2000; Minkevich et al., 2010; Tombran-Tink et al., 2005; Xu et al., 2006). The main source of PEDF in the ocular tissue is retinal pigment epithelium (Tombran-Tink and Barnstable, 2003), but it is also produced by sclera fibroblasts and by the eye Tenon's capsule (TC) – an adjacent to the sclera connective tissue sheath (Jablonski et al., 2000; Shauly et al., 1992). By using transmission electron microscopy we have shown previously that the pathological changes occurring in the TC extracellular matrix in myopic eyes are similar to the defects observed in the sclera upon advanced myopia (Iomdina et al., 2008).

In contrast to the sclera, TC samples are easier to collect during various surgical procedures without harm to the patients and therefore they are most suitable material for myopia studies. Here we analyzed TC samples to shed light on the biochemical and the histological changes taking place during myopia progression. Specifically, we have demonstrated that the disruption of PEDF proteolysis can be a critical factor of abnormal metabolism in the scleral tissue in progressive myopia.

It is well known that endogenous PEDF generally migrates in the gradient SDS-PAGE as a broad band (looking like a doublet band) with apparent MW ~50 kDa (Liu et al., 2012). By using non gradient SDS-PAGE and Western blotting with anti-PEDF peptide antibodies directed against different portions of the protein we were able to demonstrate that the doublet band corresponded to 2 isoforms of PEDF: the full-length PEDF with the observed molecular weight of 50 kDa (PEDF50), and its 45 kDa isoform truncated at the C-terminus (PEDF45). The analysis of the distribution of the isoforms in the human eye TCs showed that in the normal healthy eye TCs PEDF50 and PEDF45 were present in equal amounts while dominating PEDF50 was found in TCs obtained from highly myopic patients. We hypothesize that PEDF45 is likely a product of limited proteolysis of PEDF50 at the Leu382-Thr383 bond within the sequence of the serpin reactive center loop (RCL): Thr371-Thr-Pro-Ser-Pro-Gly-Leu-Gln-Pro-Ala-His-Leu-Thr383, which is the most exposed and accessible to endogenous proteases PEDF region (Carrell et al., 1987; Simonovic et al., 2001; Steele et al., 1993).

Here we present detail studies of the normal PEDF processing in TC and its alteration in progressive myopia. Analysis of the representative sets of TCs from the control and myopic groups enabled us to establish quantitative characteristics of the PEDF normal and abnormal processing in the eye tissue as well as to evaluate the role of this process in myopia pathogenesis.

2. Materials and methods

2.1. Human subjects

188 human TC samples were collected during various surgical procedures from 122 myopic patients (10–22 y.o. age group, mean age 15.0 ± 1.3 y.o.) and from 66 patients of the control group (7–23 y.o. age group, mean age 16.5 ± 2.0 y.o.). The myopic group consisted of patients undergoing operations aimed to sclera reinforcement for arresting progressing myopia of high degree (cycloplegic refraction of −5.75 to −31.0 diopters) with or without disease complications. Based on a comprehensive ophthalmologic examination 60 myopic patients were shown to have peripheral retinal degeneration (PRD) – the degenerative changes in the periphery of the eye fundus and 62 patients had uncomplicated progressive myopia without changes in the fundus. The control group consisted of individuals without any eye pathology characterized by the normal emmetropia refraction (refraction of ±0.25 diopters) and with hyperopia of low degree (refraction of +0.25 diopters to +3.0 diopters), who received surgical treatment for strabismus or eye injuries. In patients with a low degree of hyperopia the anatomical and functional parameters of eyes were close to healthy controls, except for a small deviation in the refractive power of the eye (Benjamin and Borish, 2006). Patients with strabismus were also included in the control group since this pathology does not cause changes in the eye inner and outer membrane.

All patients (or the patient's parents or legally acceptable representative) provided written informed consent prior to any study-related activities. The study was approved by the medical ethics committee of Moscow Helmholtz Research Institute of Eye Diseases and was conducted in accordance with the Declaration of Helsinki.

2.2. Tenon's capsule samples

The TC specimens with weight of 25–50 mg were obtained during surgical operation and not later than 30 min after excision they were either subjected to the immunohistochemical and proteolytic activity experiments or frozen in liquid nitrogen. The TC samples were stored at −70 °C and thawed directly prior to the protein or RNA extraction procedures. In Western blotting (WB) and enzyme-linked immunosorbent assay (ELISA) experiments the uncomplicated and complicated by PRD myopic samples were analyzed separately, in other experiments they were joined in one group.

2.3. Extractions of soluble and insoluble protein fractions from TC

Frozen TC samples were subjected to 2× homogenization in 200 μl of 100 mM phosphate buffer, 10 mM EDTA and 1 mM PMSF and subsequently to 3× of 30 s ultrasonic treatments on ice. The homogenized samples were centrifuged at 12,000 g and 4°C for 20 min, the supernatants were taken for further analysis of soluble protein fractions and the pellets were used for extraction of insoluble proteins. Protein concentration in the supernatants was determined by Lowry method (as described in Lowry et al., 1952).

In order to collect insoluble protein fractions from TCs, the pellets were subjected to 2× homogenization in 150 μl of 100 mM phosphate buffer with 2% SDS and 2 M urea, then they were centrifuged for 20 min at 12,000 g and 4°C to remove solid material. Protein concentration in the pellets was determined by a Bio-Rad protein assay, following the producer's instructions and using 20-fold dilution.

2.4. Western blotting analysis

SDS-polyacrylamide gel electrophoresis in 10% polyacrylamide gel (SDS-PAGE) was carried out as described in Laemmli (1970). Protein samples (20 μg) from either soluble or insoluble fractions were loaded. To assure the correct molecular weight identification recombinant PEDF (Minkevich et al., 2012) was loaded on each gel. Subsequently, the protein samples were transferred into immobilon-P membrane for immune blotting (Millipore, USA). The densitometry of immunoblots was carried out by using an ImageJ software (National Institutes of Health, USA).

In WB analysis we used the following antibodies: rabbit polyclonal anti-PEDF antibody (US Biological, USA); rabbit polyclonal antibodies to PEDF peptides Lys345-Glu366 and Phe394-Asp414, previously generated in our laboratory by Dr. Irina Kostanian