Experimental Eye Research 125 (2014) 79–88

Contents lists available at ScienceDirect

Experimental Eye Research

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

Bone morphogenetic protein 2: A potential new player in the pathogenesis of diabetic retinopathy

Khaled A. Hussein [a, b, c], Karishma Choksi [a, b], Sara Akeel [a], Saif Ahmad [b, d], Sylvia Megyerdi [a], Mohamed El-Sherbiny [a, b, e], Mohamed Nawaz [f], Ahmed Abu El-Asrar [f], Mohamed Al-Shabrawey [a, b, e, g, h, *]

[a] Department of Oral Biology/Anatomy College of Dental Medicine, Georgia Reagents University Augusta (GRU_A), USA
[b] Vision Discovery Institute, Medical College of Georgia, GRU_A, USA
[c] Oral and Dental Research Division, Department of Surgery and Medicine, National Research Center, Egypt
[d] Department of Biological Sciences, Rabigh College of Science and Arts, King Abdulaziz University, Jeddah, Saudi Arabia
[e] Department of Anatomy, Mansoura Faculty of Medicine, Mansoura, Egypt
[f] Department of Ophthalmology, King Saud University, Riyadh, Saudi Arabia
[g] Department of Ophthalmology, Medical College of Georgia, GRU_A, USA
[h] Department of Cellular Biology and Anatomy, GRU_A, USA

ARTICLE INFO

Article history:
Received 7 November 2013
Accepted in revised form 15 May 2014
Available online 6 June 2014

Keywords:
diabetic retinopathy
BMP 2
VEGF
inflammation
retinal endothelial cells
leukocyte adhesion
blood–retinal barrier
Müller cells

ABSTRACT

Diabetic retinopathy (DR) is one of the most common complications of diabetes mellitus. Vision loss in DR principally occurs due to breakdown of the blood–retinal barrier (BRB), leading to macular edema, retinal detachment and inner retinal and vitreous hemorrhage. Several growth factors have been shown to play crucial role in the development of these vascular changes; however, the cellular and molecular mechanisms of DR are not yet fully revealed. In the current study we investigated the role of bone morphogenetic protein-2 (BMP2) in DR. We examined the changes in the protein levels of BMP2 in human vitreous and retina in addition to the mouse retina of streptozotocin-induced diabetes. To detect the source of BMP2 during diabetes, human retinal endothelial cells (hRECs) were subjected to high glucose (HG) for 5 days and levels of BMP2 protein were analyzed in conditioned media of these cells relative to control. We also evaluated the effect of BMP2 on the levels of VEGF in cultured rat Müller cells (rMC1). In addition, we tested the pro-inflammatory effects of BMP2 by examining its effect on leukocyte adhesion to cultured hRECs, and levels of adhesion molecules and cytokines production. Finally, the effect of different concentrations of BMP2 on permeability of confluent monolayer of hRECs was evaluated using FITC-Dextran flux permeability assay and by measuring Transcellular Electrical Resistance (TER) using Electric Cell-substrate Impedance Sensing (ECIS).

Our results show, for the first time, the up-regulation of BMP2 in diabetic human and mouse retinas in addition to its detection in vitreous of patients with proliferative DR (72 ± 7 pg/ml). *In vitro*, hRECs showed upregulation of BMP2 in HG conditions suggesting that these cells are a potential source of BMP2 in diabetic conditions. Furthermore, BMP2 induced VEGF secretion by Müller cells *in-vitro*; and showed a dose response in increasing permeability of cultured hRECs. Meanwhile, BMP2 pro-inflammatory effects were recognized by its ability to induce leukocyte adhesion to the hRECs, intercellular adhesion molecule-1 (ICAM-1) and upregulation of interleukin-6 and 8 (IL-6 and IL-8). These results show that BMP2 could be a contributing growth factor to the development of microvascular dysfunction during DR via enhancing both pro-angiogenic and inflammatory pathways. Our findings suggest BMP2 as a potential therapeutic target to prevent/treat DR.

1. Introduction

Diabetic retinopathy (DR) is the most common cause of blindness in persons aged 25–74 years and is a major socioeconomic

* Corresponding author. Oral Biology/Anatomy and Ophthalmology, College of Dental Medicine and Medical College of Georgia, Georgia Regents University Augusta (GRU_A), Augusta, GA 30912, USA. Tel.: +1 706 721 4278; fax: +1 706 721 9415.
E-mail address: malshabrawey@gru.edu (M. Al-Shabrawey).

http://dx.doi.org/10.1016/j.exer.2014.05.012
0014-4835/

burden in the United States (Zhang et al., 2010). Vascular injury during DR is characterized by an early stage of inflammatory response, leukostasis, hyperpermeability and capillary degeneration followed by pathological retinal neovascularization (RNV) (Zhang et al., 2011). Vision loss in DR principally occurs due to breakdown of the blood–retinal barrier (BRB), resulting in macular edema, retinal detachment and inner retinal and vitreous hemorrhage (Wilkinson-Berka et al., 2013). Several growth factors have been shown to play crucial role in the development of these vascular changes such as vascular endothelial growth factor (VEGF) (Al-Shabrawey et al., 2011), Angiopoietin (Rangasamy et al., 2011), insulin like growth factor (Smith et al., 1997). However, the underlying cellular and molecular mechanisms of DR are not yet fully elucidated.

Bone Morphogenetic Proteins (BMPs) comprise an extensive group of conserved growth factors of which over 30 members have been identified to date and constitute the largest subgroup of the Transforming Growth Factor Beta (TGFβ) superfamily (Ducy and Karsenty, 2000; Guo and Wu, 2012). BMPs were first detected in extracts of bone with abilities to direct ectopic bone formation (thus the name) (Urist, 1965; Wozney et al., 1988). Nowadays, they are known to be involved in several developmental processes, including critical paracrine roles relevant to vascular physiology and pathology, therefore, several investigators have suggested changing the name to Body Morphogenetic Proteins (Guo and Wu, 2012; Reddi, 2005; Shao et al., 2007; Wagner et al., 2010).

BMP2 could be considered the most investigated and clinically relevant member of the BMPs subgroup. It is a secreted dimeric protein that has been studied extensively in osteogenesis, whether during bone development or repair (Long, 2012; Tsuji et al., 2006). It has been shown that BMP2, 4 and 7 and their receptors (BMPRs) play essential roles during eye development (Du et al., 2010; Dudley and Robertson, 1997). Some investigators suggested that BMP signaling might play a role in retinal vascular homeostasis (Mathura et al., 2000) as well as diabetes induced-vascular dysfunction such as atherosclerosis (Csiszar et al., 2009; Derwall et al., 2012; Pi et al., 2012). The vascular effect of BMP signaling pathway has been correlated to its ability to induce oxidative stress, inflammatory and angiogenic responses (Akeel et al., 2012; Csiszar et al., 2006, 2009; Liberman et al., 2011). The relevance of BMPs in angiogenesis is illustrated by the discovery of BMP endothelial cell precursor derived regulator (BMPER), which is an extracellular regulator of BMPs required for proper BMP signaling (Kelley et al., 2009; Moser et al., 2003). Recently, the effect of BMPER has been studied in an oxygen-induced retinopathy (OIR) mouse model highlighting the role of this extracellular regulator of BMPs in modulating BMP signaling at the protein level after angiogenic stimuli (Moreno-Miralles et al., 2011). However, despite these evidences suggesting an important role of BMPs in the induction and/or maintenance of vascular inflammation and angiogenesis in pathological conditions, such as DR, the underlying mechanism still remains relatively unclear. In addition, the role of BMP2 in the pathogenesis of DR has not been yet characterized.

In the current study we investigated the role of BMP2 in DR. We examined the changes in the protein levels of BMP2 in diabetic human vitreous and retina samples, diabetic mouse retinas, in addition to, conditioned media of hRECs treated with HG. We also evaluated the effect of BMP2 on the levels of VEGF in cultured Müller cells. Finally, we assessed the effect of BMP2 on the barrier function of hRECs, on the adhesion of leukocytes to cultured hRECs and the levels of ICAM-1 in the same cells.

2. Materials and methods

2.1. Human samples

2.1.1. Retina samples

Human retina and retinal sections were obtained from the Cooperative Human Tissue Network Hospital (CHTN) of the University of Pennsylvania and Capital Bioscience (Rockville, MD).

2.1.2. Vitreous samples

Undiluted vitreous fluid samples (0.3–0.6 ml) were obtained from 11 patients with proliferative diabetic retinopathy (PDR). The indications for vitrectomy in patients with PDR were traction retinal detachment, and/or non-clearing vitreous hemorrhage. Vitreous samples were collected undiluted by manual suction into a syringe through the aspiration line of vitrectomy, before opening the infusion line. The samples were centrifuged (500 rpm for 10 min, 4 °C) and the supernatants were aliquoted and frozen at −80 °C until assay. This study was approved and conducted by the Research Centre, College of Medicine, King Saud University; in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki), and informed consents were obtained from all patients.

2.2. Experimental animals

All animal experiments followed the guidelines established by the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the Georgia Regents University. C57Bl/6J mice (Jackson Laboratories, Bar Harbor, ME), weighing between 20 and 25 g were used for the *in vivo* experiments.

Experimental diabetes was developed in 6–8 weeks old mice by intra-peritoneal injection of 55 mg/kg Streptozotocin (STZ) dissolved in sterile water for 3 consecutive days as described previously. Mice were considered diabetic when their plasma glucose level exceeded 250 mg/dL. Diabetic mice (10–12 weeks from the onset of diabetes) and age matched control mice (normal) were sacrificed using carbon dioxide (CO_2) inhalation and all efforts were made to minimize suffering. Retinas from both groups were processed for quantification of the levels of BMP2 protein in retinal homogenate and retinal frozen section.

2.3. Cell culture

2.3.1. Human retinal endothelial cells (hRECs)

Cells (hRECs, Cell Systems, Kirkland, Washington) from passages 6–8 were grown on gelatin-coated dishes and maintained in M199 Media (Life Technologies, Grand Island, NY) supplemented with 10% Fetal Bovine Serum (FBS, Atlanta Biologicals, Atlanta, GA), 1% Bacc Off® (Ciprofloxacin, Catalog #4Z0-643, Cell Systems, Kirkland, Washington) and 2% recombinant human growth factor (RocketFuel Catalog #SF-4ZR-500-R, Cell Systems, Kirkland, Washington). After the cells were 80–90% confluent, they were serum starved (1% FBS) overnight, then treated with BMP2 (50, 10 and in some experiments 5 ng/ml) or high glucose (HG) (D-Glucose, 30 nM). The osmolarity of the control group, in the HG experiment, was adjusted to the same level of HG group using L-Glucose (30 mM). Experiment was terminated after 24 h of BMP2 treatment for analysis of barrier function and leukocyte adhesion. Cell lysates and conditioned media were used for Western Blot analysis of ICAM1 and multiplex assay of various cytokines respectively.