Identification of biomarkers for diabetic retinopathy

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IDENTIFICATION OF BIOMARKERS FOR DIABETIC RETINOPATHY

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Chapter 1

Introduction

DIABETIC RETINOPATHY

Diabetes mellitus is a metabolic disorder that is characterized by high glucose levels which may result in damage to various organs, including the heart, kidneys, and eyes. Several theories by which high glucose levels causes dysfunction of these organs have been proposed, including the aldose reductase, advanced glycation endproduct, reactive oxygen intermediates, and protein kinase C theory[1]. Multiple studies have shown a relationship between the degree of glucose levels and the development of complications, including diabetic eye disease. The Diabetes Control and Complication Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS) have provided evidence that intensive glucose control reduces the risk of visual loss in patients with type 1 and type 2 diabetes, respectively[2,3]. Despite the growing understanding of the complex processes involved in diabetic complications, diabetic retinopathy (DR) remains a leading cause of vision loss in working-aged people[4]. DR is characterized by capillary closure and retinal vascular hyperpermeability. Early histological signs of DR include the loss of pericytes and capillary basement membrane thickening. These processes may lead to outpouchings of capillaries and the formation of microaneurysms[5]. Micro-aneurysms are frequently the first clinically visible hint of DR during eye examination. The number of micro-aneurysms is associated with DR progression and breakdown of the inner endothelial blood-retinal barrier (BRB), characterized by hard exudates and localized areas of retinal vascular hyperpermeability[6,7]. The leakage of fluid into the retinal tissue may exceed clearance, leading to a major cause of visual loss in diabetic patients: diabetic macular edema (DME).

DIABETIC MACULAR EDEMA

The pathogenesis of DME is not fully understood. Although the breakdown of the BRB is considered to be a major mechanism in the development of DME, other mechanisms may include capillary non-perfusion, neuronal damage, and alterations of the vitreous gel[9]. Vascular Endothelial Growth Factor (VEGF) is a major mediator of proangiogenic factors which may produce DME by altering endothelial tight junctions and transcellular flow[5]. Intraocular delivery of anti-VEGF therapies are now used widely in the treatment of DME. However, it is estimated that about 50% of DME patients are refractory to anti-VEGF therapies[10,11]. At present, robust biomarkers to identify which DME patients are potential good and non-responders to anti-VEGF therapies have not been established.

NECESSITY OF BIOMARKERS FOR DR

The global rise in diabetes will result in 552 million individuals being affected by this chronic sight-threatening disease by the year 2030[12]. By the year 2050, 1 in 3 persons in the United States are projected to have diabetes, with approximately half having some degree of DR

at any point in time[13]. Thus, ocular complications from diabetes are a major and expanding public health concern. There is a critical need to identify biomarkers for DR[14]. Although anti-VEGF agents are effective, there is a need for the substantial proportion of patients who do not respond completely to anti-VEGF therapy[11]. One difficulty that prevents the development of new treatments is the identification of robust biomarkers of DR that could help define targets for new therapies or provide more effective management strategies[15]. Another is the prolonged period needed to assess changes in vision and progression of disease by fundus photography. Associations found to date are generally not sensitive or specific enough to be reliable biomarkers for DR[16].

IMAGING BIOMARKERS FOR DR

Imaging techniques in DR include fundus photography, fluorescein angiography (FA) and optical coherence tomography (OCT). FA visualizes areas of retinal hyperpermeability, but these areas are difficult to quantitate and often show a low correlation with retinal thickening on OCT[17]. Retinal features visualized by noninvasive imaging techniques such as OCT may be potential candidates for biomarkers of DME. The definition of a biomarker is 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention'[18]. They may be part of the pathophysiological pathway of the endpoint of interest[19]. OCT generates high-resolution cross-sectional images by measuring the echo time delay and magnitude of back-reflected light. It is a noninvasive imaging modality that allows quantitative measurements of retinal thickness, as well as evaluation of anatomic lesions different from retinal thickening[20]. Specific OCT patterns have been reported to be predictive of outcome after anti-VEGF therapy. Patients with cystoid macular edema (CME) pattern have been found to achieve better visual acuity and greater changes in retinal thickness after anti-VEGF therapy, while patients with diffuse pattern have been reported to exhibit a less favorable outcome[21-23]. In addition to specific OCT patterns, anatomic characteristics such as the presence of highly reflective spots, disruption of inner/outer retinal layers and intraretinal cysts have been suggested to be relevant features in predicting the response of DME patients to anti-VEGF therapies[24-28].

NOVEL BIOMARKERS FOR DR

Aiello, King, and colleagues have identified VEGF as an important mediator in the development of DME by proteomic analysis of the vitreous fluid of patients with DR[33]. Other potential mechanisms and biomarkers of DR may be found in other and more accessible fluids, such as the peripheral blood of patients with DR. The increased understanding of the

processes involved in DR has offered approaches to select candidate circulating biomarkers based on pathogenic mechanism and structural damage. Recently, a number of circulating biomarkers associated with pathogenic mechanisms and structural retinal tissue damage of DR have been independently and significantly associated with DR[34-39]. Endothelial progenitor cells are circulating cells that have been suggested to play roles in the regeneration of vessel damage and may be involved in the pathogenesis of DR[35,36,40,41]. Other candidate biomarkers are related to structural damage of the retina in DR[34,38,39,42]. However, little is known about the potential value of these biomarkers in patients with DME. In addition to further research investigating the potential of these biomarkers, it is essential to investigate potential VEGF-independent mechanisms of DME, such as the plasma kallikrein kinin system (KKS).

KALLIKREIN KININ SYSTEM

The KKS has been discovered by Feener and colleagues as a potential mediator of BRB loss and VEGF-independent mechanism of DME[43-49]. Plasma kallikrein is activated by the coagulation factor XIIa, which is released by the contact system during inflammation and bleeding[47]. Once generated, plasma kallikrein divides high molecular weight kininogen to produce bradykinin, which can stimulate vasoactive hormones (e.g. nitric oxide) that influence the BRB[48]. Components of the KKS, including plasma kallikrein and factor XII, have been identified in the vitreous fluid of patients with DME[44]. These findings suggest that components of the KKS in plasma may be potential biomarkers in patients with DME.

AIM OF THE THESIS

The aim of the thesis is to identify potential novel biomarkers for DR. Identification of potential novel biomarkers for DR could contribute to the definition of targets for new therapies and provide more effective management strategies for DR.

OUTLINE OF THE THESIS

Components of the KKS may be potential biomarkers for DR. In Chapter 1a, we review the role of the KKS in diabetes and the mechanisms that contribute to KKS activation. The physiological actions of components of the KKS are described. The review provides an overview of the factors that have been associated with activation of the KKS and the functions that have been supported by clinical data. In addition, genetic studies of the KKS gene and the biochemical functions are summarized. Future directions and new strategies to monitor KKS activity are also discussed. As discussed above, the biological effects of the KKS are primarily

mediated by bradykinin. Chapter 1b characterizes the effects of bradykinin on retinal structure and proteome of the rat retina. This chapter concludes with Chapter 1c, which examines the associations between DR, kidney disease, and cardiovascular disease by characterizing individuals with type 1 diabetes in the United States and Finland.

The aim of study in Chapter 2 was to examine potential imaging biomarkers for DR. In chapter 2a, we examine the predictive value of OCT patterns and retinal features on visual outcomes and retinal thickness of patients with DME receiving anti-VEGF treatment. This is important, since there are no methods available in clinical practice to assess which patients with DME are good-responders and non-responders to anti-VEGF therapy. Within the prospective, multicenter BRDME (comparing the effectiveness and costs of Bevacizumab to Ranibizumab in patients with Diabetic Macular Edema) study, we evaluated the potential of retinal features visualized by OCT as biomarkers in patients with DME that receive anti-VEGF therapies. Although diabetic neuroretinal abnormalities have been previously described, such as thinning of inner retinal layers[50], changes in individual retinal layers have not been fully explored across multiple decades of diabetes duration. In chapter 2b, we assessed retinal layer thicknesses on OCT on 1413 eyes of 776 patients across a wide range of age, duration of diabetes, and DR severity.

In Chapter 3a, we determined whether specific circulating biomarkers could be used as potential markers for patients with DME that receive anti-VEGF therapy. The candidate biomarkers were selected based on literature on structural damage and pathogenic mechanism of DR and included mRNA levels of CD34+, CD133+, rhodopsin, RPE65, and retinoschisin. Blood samples were collected at baseline in a cohort of patients from the BRDME study and OCT images and visual acuity were analyzed during anti-VEGF treatment to assess the associations between candidate markers and changes in retinal thickness and visual acuity.
Chapter 3b focuses on the retinal and vitreous protein profiles from participants of the Joslin 50-Year Medalist Study. The Joslin Medalist Study characterized over 1000 people with type 1 diabetes duration of 50 years or more. Over 35% of these individuals exhibit only no-mild diabetic retinopathy (DR), independent of glycemic control, suggesting the presence of endogenous protective factors against DR. The goal of the study was to identify these protective factors with a high therapeutic potential to prevent or stop the progression of DR.

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Chapter 2

Role of Kallikrein Kinin System and Common Factors in Diabetes Complications