Diabetic Retinopathy (DR) is one of the most common eye diseases which can lead to partial or complete blindness. Results from our lab have shown that early stages of DR involve the infiltration of monocyte-derived macrophages which secrete Transforming Growth Factor beta-1 (TGFβ1). We have also reported that TGFβ1 upregulates a BIGH3 protein (TGFβ1-Induced Gene Human Clone 3) which induces loss of retinal endothelial cells by apoptosis. Previous studies also indicate that C-terminus of BIGH3 is involved in apoptosis which can interact with specific integrins. We hypothesize that the clustering of C-terminus (EPDIM or RGD) of BIGH3 to a specific integrin (a3b1, a laminin receptor or a5b1 fibronectin receptor) in the ECM is required for inducing apoptosis. The objective of current study is to identify integrins expressed in Rhesus Monkey endothelial cells which may have a possible role in BIGH3 mediated apoptosis.

Introduction

Diabetic Retinopathy affects people all over the world. People with Diabetic Retinopathy have either type 1 or type 2 diabetes. Although there are ways to treat or slow down Diabetic Retinopathy vision that is lost cannot be recovered.[3] In earlier reports it was discovered that RGD or EPDIM is a mediator in the activation of apoptosis. We have decided to discover what integrin will bind to the mediator that will activate apoptosis.

Methods

-Rhesus Retinal Endothelial Cells (RhREC) were purchased from ATCC (Cat No: CRL-1780, RF/6A). The cells were transferred at an early passage number and were maintained in a 275 Flask in MEM media (10% FBS).

-RhREC lysate was generated using RIPA buffer and BCA assay (Thermo Scientific catalog no: 23225) was performed according to manufacturer instructions to determine lystate concentration and Western Blotting was used to determine a3 and a5 protein levels in lysates.

-Reverse Transcription PCR: RNA was isolated using Qiagen Rneasy kit (Qiagen, catalog no: 74104). RNA was then reverse transcribed into cDNA using Taqman Reverse Transcription reagents (Life Technologies, Cat No: N8080234). Quantitative PCR was used to measure the mRNA using Syber Green PCR MIX (Life Technologies, catalog no: 4334973). The comparative Ct method was used to obtain quantitative data.

Results

-qPCR

 Alpha 3

 BCA Plate

 RhREC cells

 Western blotting

 RhREC cells passage 30. Magnification 10x

 RhREC

 BCA Plate

 Result: 5.196

 RhREC

 Western Blot

 Lane 1

 Lane 2

 Lane 3

 Lane 4

 Lane 5

 Lane 6

 Lane 7

 Alpha 3

 Alpha 5

 Alpha 3 does not appear to be conclusive. The band did not appear in the location where a positive confirmation could have been made.

 Alpha 5

 The band from alpha 5 does not can not be well observed and therefore can not be determined.

References


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