J Biochem Tech (2012) 4(1): 565-567
ISSN: 0974-2328

Analysis of diabetic retinopathy biomarker VEGF gene by computational approaches

Jayashree S, Ramesh N, Vinni Viridi, Shivaprasad, Vijayalakshmi K

Received: 29 August 2012 / Received in revised form: 01 July 2013, Accepted: 01 July 2013, Published online: 25 June 2013

Abstract

Diabetic retinopathy, the most common diabetic eye disease, is caused by changes in the blood vessels of the retina which remains the major cause. It is characterized by vascular permeability and increased tissue ischemia and angiogenesis. One of the biomarker for Diabetic retinopathy has been identified as Vascular Endothelial Growth Factor (VEGF)gene by computational analysis. VEGF is a sub-family of growth factors, the platelet-derived growth factor family of cystine-knot growth factors. They are important signalling proteins involved in both vasculogenesis and angiogenesis, Over expression of VEGF can cause vascular disease in the retina of the eye and other parts of the body. Drugs can inhibit VEGF and control or slowdown those disease. Computational analysis of VEGF with other genes responsible for diabetic retinopathy were done by aligning those genes by pair wise and multiple sequence alignments. MSA shows VEGF's role in diabetic retinopathy and its related with other genes and proteins responsible for pathogenesis of diabetic retinopathy. Also the determination of the promoter and conserved domain of VEGF gene help us to identify its expression levels. Thus molecular docking studies were carried out to analyse the biomarker VEGF, that helps in treatment of diabetic retinopathy which is proliferative in nature due to uncontrolled angiogenesis.

Keywords: VEGF , Diabetic retinopathy, Bioinformatics analysis ,molecular docking.

Introduction

In retinal diseases such as Diabetic Retinopathy vision loss is due to retinal vascular dysfunctions. (Tapp RJ, 2003) Angiogenic stimulators and angiogenic inhibitors play important role in regulation of vascular functions. (Chung SS 2005), Under normal conditions balance is maintained between stimulators and Inhibitors. During pathological conditions like Diabetic Retinopathy this balance is disturbed due to the overproduction of angiogenic stimulators and decreased production of angiogenic inhibitors. Vascular Endothelial Growth Factor (VEGF) major angiogenic factor play a critical role in normal and pathological angiogenesis. (A A Rao2008.)

Vascular endothelial growth factor (VEGF) is a signal protein, a 45-kDa homodimeric glycoprotein produced by cells, a major mediator of vascular permeability and angiogenesis, may play a pivotal role in mediating the development and progression of diabetic retinopathy. (Geraldes 2009; Way K J 2002) VEGF is produced from many cell types within the eye, and past studies have shown that VEGF levels are markedly elevated in vitreous and aqueous fluids in the eyes of individuals with proliferative retinopathy (Wilkinson 2004)

Significant up regulation of VEGF expression at both RNA & Protein levels suggesting inhibitory effect of VEGF binding to retinal capillary epithelial cells controlling its expression at transcription level. Thus VEGF a biomarker for diabetic retinopathy is analysed using bio informatics tools. (A. A Rao 2008; S Gedela, 2007; Pardianto G 2005)

Methodology

The genes responsible for diabetic retinopathy were retrieved from NCBI in FASTA format. About 25 to 30 significant genes were considered.

Among them the VEGF-A "NM_001204385.1" was identified as a significant one. And similarity searches through blast with VEGFA was done and six includes other VEGFA and related sequences showing around 100% similarity were taken for sequence analysis as listed in the table 1.

Complete analysis of VEGFA and its related sequences were carried out by using the following bioinformatics tools (Table 2) which help VEGFA to consider as a biomarker for diabetic retinopathy.

The first six sequences from the blast output were selected based on the percentage similarity and uploaded for Multiple Sequence

Jayashree S*, Ramesh N, Vinni viridi, Shivaprasad

Reva Institute of Science and Management, Bangalore

*Email:- jayashree1acharya@gmail.com

Vijayalaskhmi K

Bharathi Women's college, Chennai, India

Alignment (MSA) along with the VEGFA NM_001204385.1. The closely related ones of MSA output sequences were again analyzed by pairwise alignment.

Table 1: VEGFA and related sequences.

NCBI Accessions	Description
AB209485.1	Homo sapiens mRNA for vascular endothelial growth factor variant protein
NM_001171630.1	Homo sapiens vascular endothelial growth factor A (VEGFA), transcript variant 8, mRNA
NM_001033756.2	Homo sapiens vascular endothelial growth factor A (VEGFA), transcript variant 7, mRNA
CR614384.1	Full-length cDNA clone CS0DM005YB14 of Fetal liver of Homo sapiens (human)
AF095785.1	Homo sapiens vascular endothelial growth factor (VEGF) gene, promoter region and partial cds
AF437895.1	Homo sapiens vascular endothelial growth factor (VEGF) gene, partial cds:

The structure and function of each gene and their complexities of promoters will be critical for developing the most effective diagnostic techniques and disease treatments. Promoter analysis for VEGFA "NM_001204385.1" was done by TSSG software tool. And the prediction of the structure of protein was done by Swiss Model Repository. Additionally, biomarker studies of VEGFA were done by identification of tandem repeat element with the help of TRF repeat finder in the VEGFA related sequences.

The docking studies were done using online tool (Cluspro 2.0, (Structural Bioinformatics Lab, Boston University, USA).

Table 2: - Tools used in the methodology.

Programme	Tool	Website
Sequence isolation	Database and FASTA	www.ncbi.nlm.nih.gov
Multiple sequence alignment	clustalW	www.ebi.ac.uk/clustalw
Pairwise sequence alignment	EMBOSS	www.ebi.ac.uk/Tools/emboss/align
Conserve sequence	BLAST	www.ncbi.nlm.nih.gov/blast
Gene promoters	TSSG	www.softberry.com/berry.tssp&group=programs&subgroup=promoter
Proteins Data	PDB	www.rcsb.org
Tandem repeats	TRF finder	http://tandem.bu.edu/trf/trf.submit.options.html
Docking	Clusproprotein-protein dock.	Cluspro.bu.edu

Results

From the MSA results of VEGF genes, NM_001204385.1 sequence showed close relation with the other VEGFA genes as shown in the phylogenetic tree and AF437895.1, AF095785.1 and AB209485.1 are under the same cluster.

Phylogenetic tree

The pairwise alignment of very closely related sequence of VEGFA are NM_001204385.1 and NM_ 00171630 with 99% similarity.

The biomarkers of VEGFA sequence (NM_001204385.1) were found by identifying the promoter element and tandem repeats. The promoter identified by TSSG software shows a single transcription factor binding sites at position - 581 and the tandem repeats finder results were shown in Table 3.

Table.3: The various regions of tandem repeats of the other VEGF genes

NCBI Accession numbers	Regions of tandem repeats
AB209485.1	8278-8460,16607-10674,10609-10633,10619-10682
NM_001171630.1	1811-1878,1813-1837,1823-1886.
NM_001033756.2	1907-1974,1909-1933,1919-1982.
AF437895.1	10327-509,12656- 682, 12668-12731

Prediction of tertiary structure by Swiss model

Prediction of tertiary structure of the VEGFA sequence (NM_001204385) is done through Swiss Model Repository.

The Swiss Model Repository works on the basis of assessing protein structures with a non-local atomic interaction energy. Accessing protein structures is carried out considering the following parameters like C-beta interaction energy, All-atom pairwise energy, torsion angle energy, secondary structure agreement, solvent accessibility agreement together with their scores with respect to scores obtained for high-resolution experimental structures of similar size solved by X-ray crystallography. Predicted structure of VEGFA sequence using Swiss Model Repository is shown in the Figure 1.

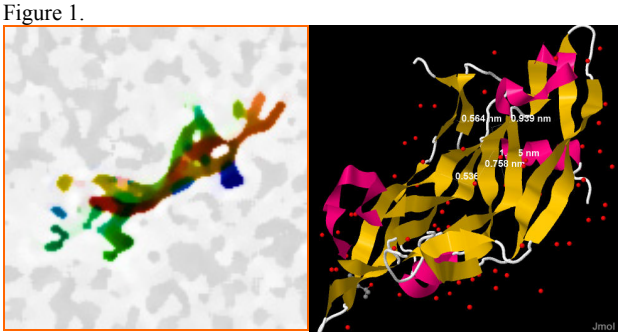

Figure 1: Predicted structure of VEGFA sequence (NM_001204385) using Swiss - Model Repository

Docking studies by inhibitors

VEGF is a promising target for anti angiogenic theraphy. Inhibitors of angiogenesis are endostatin, angiotensin, arrestin and tumstain. These are naturally occurring proteins that inhibit the proliferative angiogenesis in proliferatie diabetic retinopathy (Rao 2008). Considering, VEGF as receptor and angiotensin and endostatin as ligands, protein-protein docking studies were done by ClusPro 2.0 (Structural Bioinformatics Lab, Boston University, USA). The docking results were shown in Figure 2.

1. The *cluspro* docking program generates docked images by rotating the ligand with 70,000 rotations. For each rotation, it translates the ligand in x, y, z relative to the receptor on a grid. It choose the 1000 rotation/translation combinations that have the lowest score. These images infer the possibilities of inhibition by the inhibitor protein by blocking the hot residues of the VEGF. Thus blocking the activity of the VEGF angiogenesis in the proliferative diabetic retinopathy are shown in Fig 2B.

Conclusion

VEGF gene has been identified as a biomarker for diabetic retinopathy. These studies along with molecular protein dynamics helps us towards better understanding of the inhibitor action on VEGFA. Inhibitor studies using computational tools and in-vivo

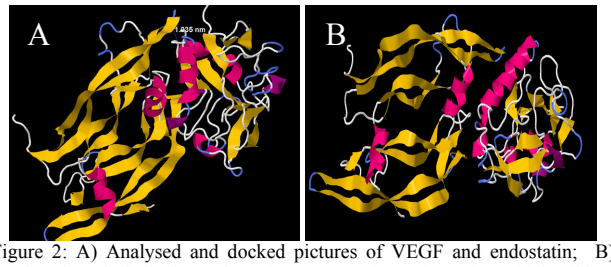

Figure 2: A) Analysed and docked pictures of VEGF and endostatin; B) Analysed and docked results of VEGF and Angiotensin. Thick ribbon represents VEGF and thin represents endostatin in both Fig 2A & 2B.

experiments will surely help the mankind in development of novel treatment techniques for diabetic retinopathy.

References

Boucher J, Masri B, Daviaud D et al (2005) Apelin, a newly identified adipokine up-regulated by insulin and obesity. Endocrinology 146 (4):1764–7171.

Dray C, Knauf C, Daviaud D et al (2008) Apelin stimulates glucose utilization in normal and obese insulin-resistant mice. Cell Metab 8 (5): 437–45.

Geraldes, Pedro, Hiraoka-Yamamoto et al. (2009) Activation of PKC-δ and SHP-1 by hyperglycemia causes vascular cell apoptosis and diabetic retinopathy. Nature Medicine 15 (11): 1298–1306

Gedela S, AA Rao, N.R Medicherla (2007) Identification of Biomarkers for type II diabetes and its complications: A bioinformatics approach. Int J Biomed Sci 3(4):229

Kertes PJ, Johnson TM, ed (2007). Evidence Based Eye Care. Philadelphia, PA: Lippincott Williams & Wilkins 6964-6967

Koonin EV (2001) Computational Genomics, National Center for Biotechnology Information, National Library of Medicine, NIH (PubMed ID: 11267880)

Kwok S Chong Roy S Gardner, Euan A Ashley, Henry J Dargie & Theresa A McDonagh (2007) Emerging role of the apelin system in cardiovascular homeostasis, Biomarkers in Medicine 1(1):37-43

Maria Sörhede Winzell, Caroline Magnusson and Bo Ahrén (2005) The apj receptor is expressed in pancreatic islets and its ligand, apelin, inhibits insulin secretion in mice. Regul Pept 131(1-3):12-7

Pardianto G et al. (2005) Understanding diabetic retinopathy. Mimbar Ilmiah Oftalmologi Indonesia 2: 65–66

Rao AA, SP Akula, H Thota, S Gedela (2008) Bioinformatics analysis of alzheimer's disease and type II diabetes mellitus: A bioinformatics approach. J Proteomics & Bioinformatics, Vol 1 ISCA

Sörhede Winzell M, Magnusson C, Ahrén B (2005) The apj receptor is expressed in pancreatic islets and its ligand, apelin, inhibits insulin secretion in mice. Regul Pept 131 (1-3):12–7

Tapp RJ, Shaw JE, Harper CA, et al. (2003) The prevalence of and factors associated with diabetic retinopathy in the Australian population. Diabetes Care 26 (6): 1731–1737

United States National Library of Medicine. 15 September 2009. Causes and Risk Factors. Diabetic Retinopathy http://nihseniorhealth.gov/diabeticretinopathy/causesandriskfactors/02.html.