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 Rao 2008.)

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 bioinformatics 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
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.

<table>
<thead>
<tr>
<th>NCBI Accessions</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB209485.1</td>
<td>Homo sapiens mRNA for vascular endothelial growth factor variant protein</td>
</tr>
<tr>
<td>NM_00117630.1</td>
<td>Homo sapiens vascular endothelial growth factor 1B (VEGFA), transcript variant 8, mRNA</td>
</tr>
<tr>
<td>NM_001033756.2</td>
<td>Homo sapiens vascular endothelial growth factor 1B (VEGFA), transcript variant 7, mRNA</td>
</tr>
<tr>
<td>CR614384.1</td>
<td>Full-length cDNA clone CSODM005YB14 of Fetal liver of Homo sapiens (human)</td>
</tr>
<tr>
<td>AF905785.1</td>
<td>Homo sapiens vascular endothelial growth factor (VEGF) gene, promoter region and partial cds</td>
</tr>
<tr>
<td>AF437895.1</td>
<td>Homo sapiens vascular endothelial growth factor (VEGF) gene, partial cds</td>
</tr>
</tbody>
</table>

The pairwise alignment of very closely related sequence of VEGFA are NM_001204385.1 and NM_00117630 with 99% similarity.

Phylogenetic tree

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.

<table>
<thead>
<tr>
<th>Programme</th>
<th>Tool</th>
<th>Website</th>
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</thead>
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<tr>
<td>Multiple sequence</td>
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<tr>
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<tr>
<td>Docking</td>
<td>Clusproproteinn-protein</td>
<td>Cluspro.bu.edu</td>
</tr>
</tbody>
</table>

Table 2: - Tools used in the methodology.

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

<table>
<thead>
<tr>
<th>NCBI Accession Numbers</th>
<th>Regions of tandem repeats</th>
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<tr>
<td>AB209485.1</td>
<td>8278-8460,16607-10674,10609-10633,10619-10682</td>
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<tr>
<td>NM_00117630.1</td>
<td>1811-1878,1813-1837,1823-1886.</td>
</tr>
<tr>
<td>AF437895.1</td>
<td>10327-509,12656-682,12668-12731</td>
</tr>
</tbody>
</table>

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 therapy. Inhibitors of angiogenesis are endostatin, angiotsin, arrestin and tumstatin. These are naturally occurring proteins that inhibit the proliferative angiogenesis in proliferative 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.

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

References

identified adipokine up-regulated by insulin and obesity.

utilization in normal and obese insulin-resistant mice. Cell
Metab 8 (5): 437–45.

Geraldes, Pedro, Hirnoka-Yamamoto et al. (2009) Activation of
PKC-δ and SHP-1 by hyperglycemia causes vascular cell
1298–1306

Biomarkers for type II diabetes and its complications: A

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 Ahren (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

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

is expressed in pancreatic islets and its ligand, apelin, inhibits
insulin secretion in mice. Regul Pept 131(1-3):12–7

factors associated with diabetic retinopathy in the Australian
population. Diabetes Care 26 (6): 1731–1737

Causes and Risk Factors. Diabetic Retinopathy
http://nihseniorhealth.gov/diabeticretinopathy/causesandriskfact
ors/02.html.