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Investigation of BDNF gene expression level by analysis of dysregulation of miRNA-15a: An *in-silico* approach

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Abstract: ***Type 2 Diabetic retinopath (T2DR) remains the leading cause of vision loss and preventable blindness in adults aged 20–74 years, particularly in middle-income and high-income countries. The pathogenesis of T2DR is a predominant cause of visual impairment and a range of hyperglycemia linked pathways have been implicated in the initiation and progression of this condition. Apparently in the past T2DR was solely considered a vascular disease as opposed to the present time where it is recognised as a neuro-vascular disease. Some pro-survival neurotrophins such as brain derived neurotrophic factor (BDNF) are considered to guard retinal ganglion and amacrine cells from degenerative. Significant reduction in the levels of BDNF have been witnessed in diabetic patients as well as animal models. miRNAs are a group of 21-23 nucleotide long, highly conserved sequences of endogenous RNAs that do not encode for any protein. Researches carried out over the last decade gives plenty of proof about the miR-15a importance in T2DR. Henceforth, miR15a could be used for the experimental purposes. miRNAs can be considered as an efficient biomarker as they maintain their stability and utility over rigorous processing phases and the presence of quantitative detection boosts their therapeutical significance.***

Keywords: Type 2 Diabetic Retinopathy, Neurodegeneration, BDNF, Prognosis, Biomarker, miR-15a, Bioinformatics

I. INTRODUCTION

T2DR is the most prevalent microvascular complication, the pervasiveness of which increases with the term of diabetes, with a general rate of up to 30% and a high risk of extreme visual impairment in 10% of subjects [1, 2]. It is a significant risk factor for T2DR and induces multiple biochemical changes, including inflammation and endothelial dysfunction in the retina [3]. Without proper treatment, T2DR advances from mild, non-proliferative T2DR to extreme non-proliferative T2DR, before the occurrence of proliferative T2DR (development of unusual new retinal veins takes place). Simultaneously, at any phase of retinopathy, with exudation and oedema at the macula of the eye, patients may likewise develop diabetic macular oedema (DME) [4, 5]. While vascular changes are the most visible clinical features in T2DR, indications of neuropathy, for example, degeneration of the inner nuclear and ganglion cell layer, and neurotic changes in the nerve fiber layer have been emphasised in the early literature on T2DR[6]. Some pro-survival neurotrophins such as brain derived neurotrophic factor (BDNF) are considered to guard retinal ganglion and amacrine cells from degenerative processes [7]. Significant reduction in the levels of BDNF have been witnessed in diabetic patients as well as animal models [8, 9]. A decreased BDNF levels in serum from diabetic patients and in diabetic animals in various studies. A decreased level of BDNF was associated with insulin resistance, decreased glucose and lipid metabolism and increase in food intake [10, 11]. Few studies among the animal models of diabetes revealed that a reduced levels of BDNF in the diabetic retina may impair neurons, thus leading to neurodegeneration [12, 13]. However, the importance of BDNF in DR is not fully illustrated. The regulation of appropriate level of BDNF in the diabetic retina may be a hopeful therapeutic target to shield neurons [14].

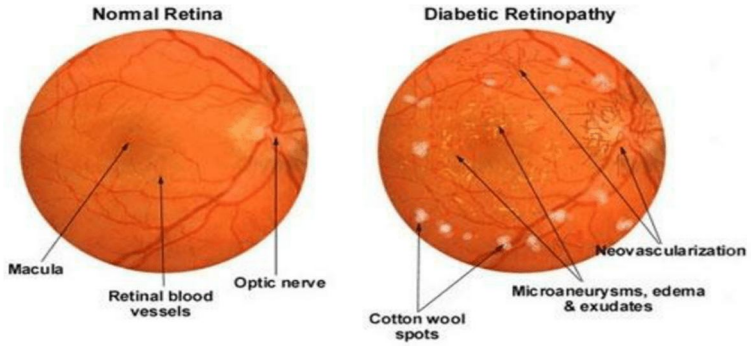


Fig 1: Normal retina Vs Diabetic retinopathy [15]

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miRNAs are a group of 21-23 nucleotide long, highly conserved sequences of endogenous RNAs that do not encode for any protein. miRNAs are known to modulate gene expression by transcriptional and post-transcriptional regulation, bringing mRNA degradation or inhibition of protein translation by binding to target genes at the seed region on the 3′-UTR . These are expressed in all human cell types and are involved in important biological processes such as cell growth, differentiation, and apoptosis [16, 17 and 18].

The expression of several mRNAs can be modulated by a single miRNA, more than 60% of mRNAs are expected to have binding sites for multiple miRNAs, hence permitting simultaneous interaction with multiple miRNAs [19]. Therefore, it is expected that the function and biogenesis of miRNAs can be modulated (modification is linked with a widespread range of human diseases, including chronic conditions [20].

The field of bioinformatics, or systems biology, which is the merging of the computational and biological science disciplines, has been an important tool for the organisation and analysis of the vast amount of biological data. The main aim of bioinformatics is to find key biological information hidden amongst a mass of raw data to identify important trends and patterns which would eventually lead to novel biomarker discovery for both diagnostic and therapeutic purpose [21].

II. MATERIALS AND METHODS

Computer with high speed internet access and various online bioinformatics tools were used.

A. miRNA-15a sequence retrieval using miRBase

miRBase is a major database for microRNA (miRNA) sequence information. miRBase plays a role in explaining the nomenclature for miRNA genes and assigning names to unique miRNAs for publications. The online miRBase database consists of published miRNA sequences, along with textual annotation and relations to the primary literature and to other secondary databases. The database supplies wide range of methods to search the data, by specific search of sequences and relative text and literature [22].

B. MirRNA-15a Sequence Analysis Using BLAST

The Basic Local Alignment Search Tool (BLAST) algorithm is a program manual that works on the basis of some smart shortcuts to perform the search faster. BLAST executes “local alignments. BLAST searches regions of local similarity between various sequences.

The program compares and analyses nucleotide or protein sequences to sequence databases and determines the statistical significance of matches. BLAST finds application in inter functional and evolutionary relationships between sequences as well as help to identify members of gene families (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Most of the proteins are modular in nature, with functional domain often being repeated within the same protein. The Blast algorithm devised as such to find these domains or shorter stretches of sequence similarity.

The local alignment approach also means that an mRNA can be aligned with a piece of genomic DNA, as is frequently required in genome assembly and analysis. If either BLAST started out by attempting to align two sequences over their entire lengths (known as a global alignment).fewer resemblances would be detected [23].

C. Search and Predict miRNA Target Using miRmap

miRmap, which for the first time comprehensively covers all four approaches using eleven predictor features, three of which are novel. This allowed us to examine feature correlations and to compare their predictive power in an unbiased way using high throughput experimental data from immunopurification, transcriptomics, proteomics, and proteomics and polysome fractionation experiments. Overall, target site accessibility appears to be the most predictive feature. The novel feature based on PhyloP, which evaluates the significance of negative selection, is the best performing predictor in the evolutionary category. miRmap combined all the features into an integrated model that almost doubles the predictive power of TargetScan [24].

D. Prediction of miRNA Regulatory Network using ComiRNet

ComiRNet is a database of miRNA target predictions and predicted miRNA regulatory networks. ComiRNet stores approximately 5 million predicted interactions between 934 human miRNAs and 30,875 gene transcripts (mRNAs) which are exploited in the construction of the hierarchies of overlapping biclusters representing potential miRNA regulatory networks [25].

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E. Functional Protein Association Network using STRING

STRING home page was opened by using link https://string-db.org/.Multiple proteins option was selected on the home page. Under list of names option, gene list was pasted. Clicked search. Then on the next page, Homo sapiens option was clicked. Clicked continue. STRING result was displayed and interpreted. Clustering was done by clicked clusters option (K-means clustering). Clustering result was displayed and interpreted [26].

F. Pathway Enrichment Analysis of miR-15a Target Gene BDNF in T2DR by KEGG Database

KEGG is worldwide used as a reference database for association and interpretation of largescale datasets generated by genome sequencing and other high-throughput experimental technologies. In addition to maintaining the aspects to support basic research, KEGG is being expanded towards more practical applications associating human diseases, drugs, and other health-related substances. KEGG is designed and explored by Kanehisa Laboratories [27].

III. RESULTS AND DISCUSSIONS

A. MicroRNA-15a Sequence Retrieval using miRBase



Fig 2: Mature sequence of hsa-miR-15a-5p and hsa-miR-15a-5p

Sequence and structures of miRNA sequences were needed for understanding their function. Thus, stem-loop structure (83 nucleotide miRNA precursor) of hsa-mir-15a and mature sequence of hsa-miR-15a-5p were retrieved from miRBase. The sequence retrieval was first and vital step for further analysis of sequences. Sequence retrieval helped in understanding the number of nucleotides present in the miRNA sequence and the stem loop structure of miR-15a. According to previous study [28], miRNA sequences should be of 20-25 nucleotides. In this study, miR-15a sequence obtained by miRBase is of 22 nucleotides. Thus, further analysis of sequence was done using other online tools.

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B. MirRNA-15a Sequence Analysis Using BLAST

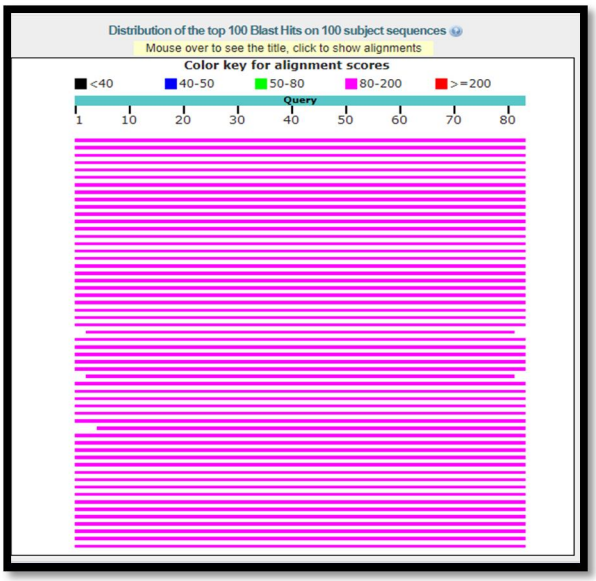


Fig 3: Graphical overview of BLAST result

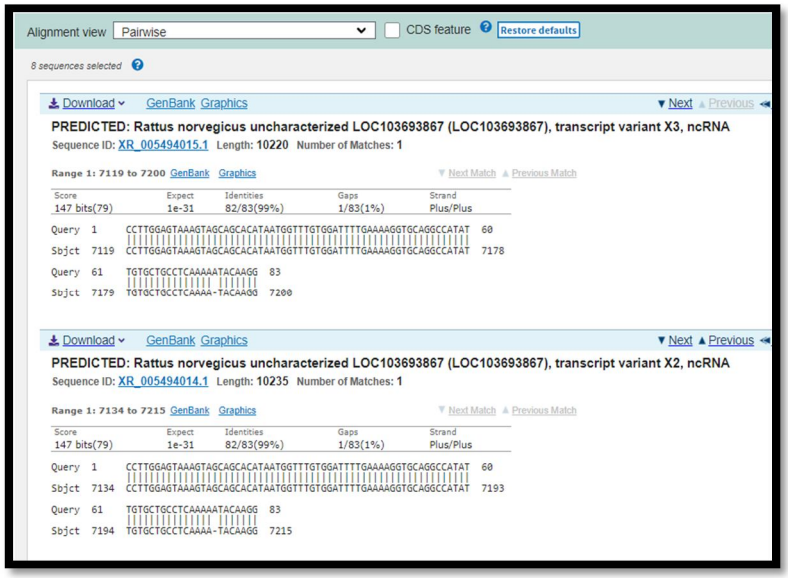
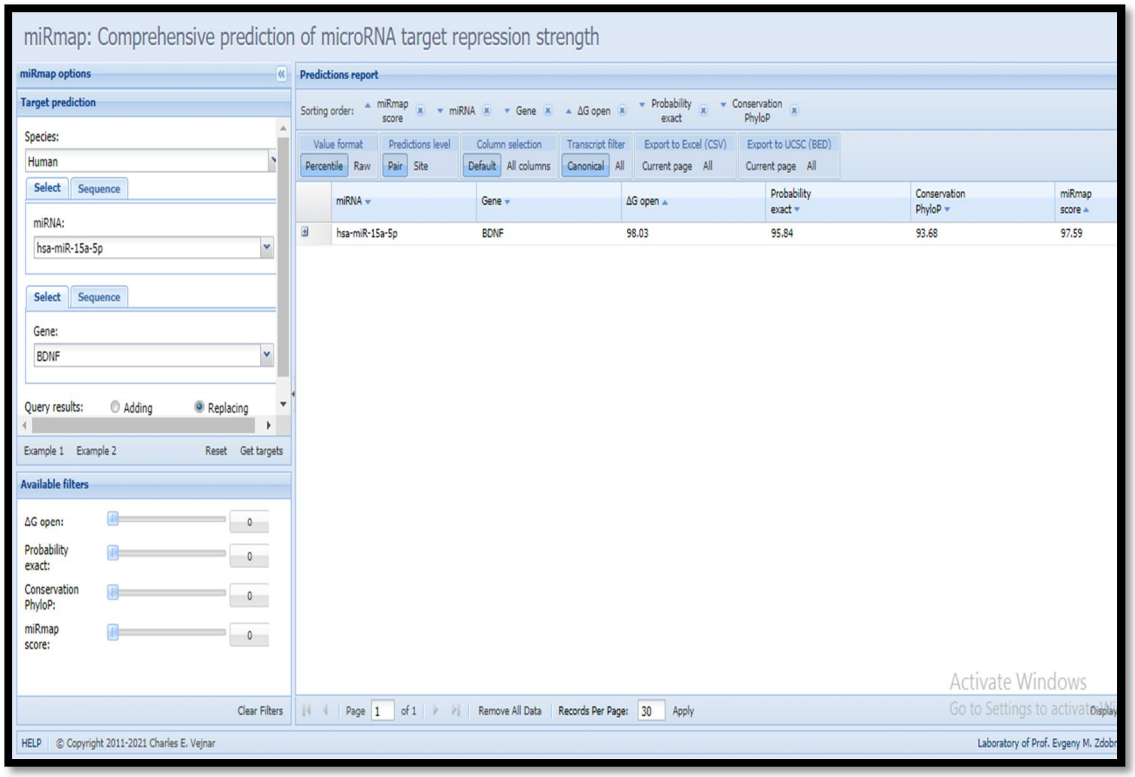


Fig 4: Pairwise sequence alignment from a BLAST report

Result demonstrated that the highest identity percentage and lowest E-value (2e-33) is of Rattus norvegicus microRNA-15a sequence (99% identity) when compared with query sequence. Therefore, results clearly indicated the conserved nature of the miR-15a among the various mammalian model organisms. It will help to infer the function of a sequence from similar sequence. Therefore, Rattus norvegicus can be used for better results for in vivo study of dysregulated miRNA-15a, which will be very helpful in identifying the changes occurring in BDNF gene in case of T2DR.

C. Search and Predict miRNA target using miRmap



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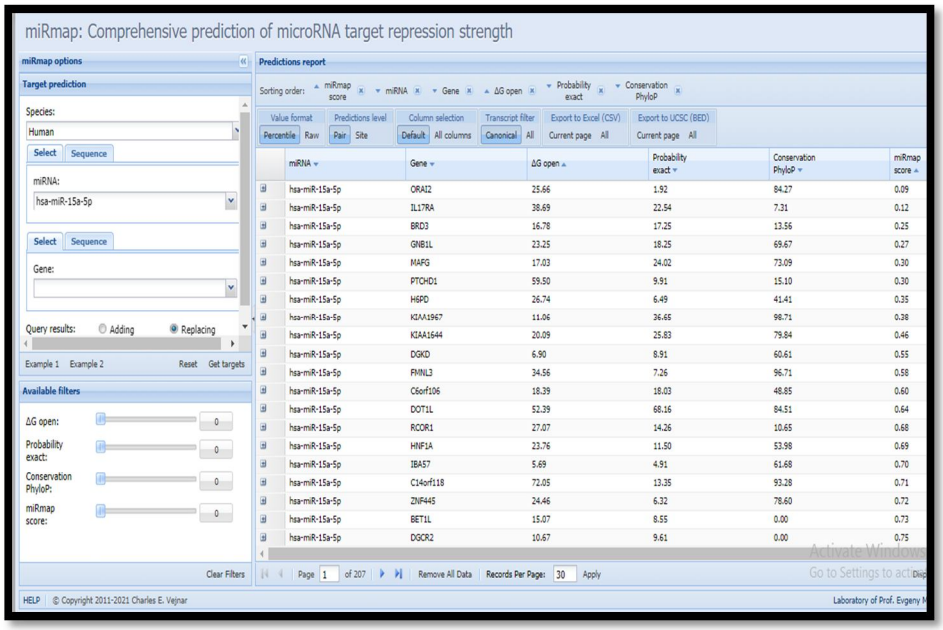
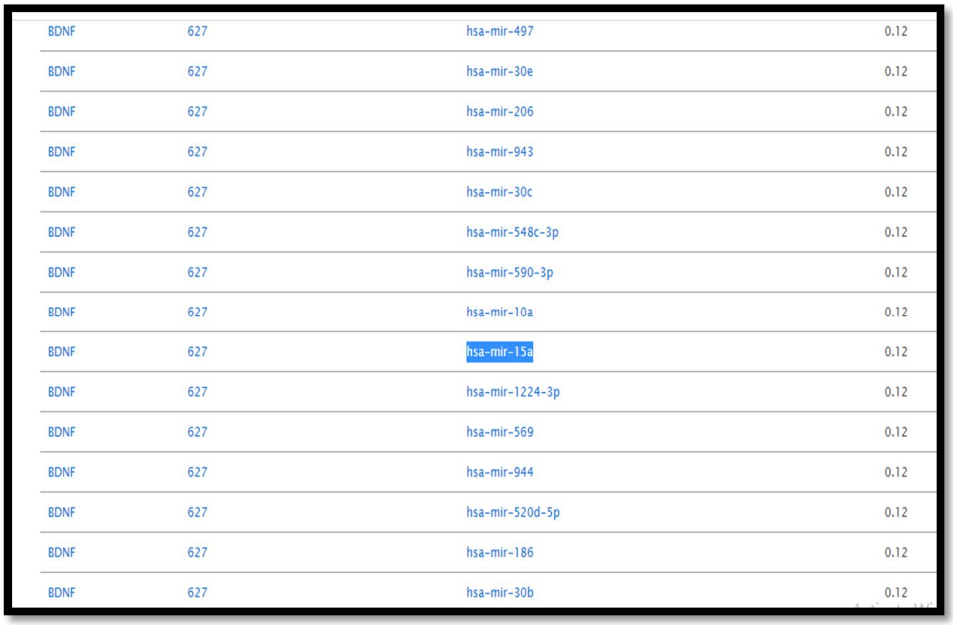


Fig 5 and 6: Target prediction between miR-15a-5p and BDNF

miRmap is used to identify the target as BDNF for the selected miRNA hsa-miR-15a-5p and it showed a free energy about 98.03, probability about 95.84 and 97.59 miRmap score. These outputs represents the suitable chances to target the selected gene. The novel feature based on PhyloP, which evaluates the significance of negative selection, is the best performing predictor in the evolutionary category. The higher value of the output represent the stronger repression.

D. Prediction of miRNA regulatory network using ComiRNet



BDNF	627	hsa-mir-497	0.12
BDNF	627	hsa-mir-30e	0.12
BDNF	627	hsa-mir-206	0.12
BDNF	627	hsa-mir-943	0.12
BDNF	627	hsa-mir-30c	0.12
BDNF	627	hsa-mir-548c-3p	0.12
BDNF	627	hsa-mir-590-3p	0.12
BDNF	627	hsa-mir-10a	0.12
BDNF	627	hsa-mir-15a	0.12
BDNF	627	hsa-mir-1224-3p	0.12
BDNF	627	hsa-mir-569	0.12
BDNF	627	hsa-mir-944	0.12
BDNF	627	hsa-mir-520d-5p	0.12
BDNF	627	hsa-mir-186	0.12
BDNF	627	hsa-mir-30b	0.12

Fig 7: miR-15a and BDNF target prediction

ComiRNET is used to identify the target as BDNF for the selected miRNA hsa-miR-15a-5p.The target as BDNF for the selected miRNA hsa-miR-15a-5p. The “search interaction” showed that the BDNF has interaction with miRNA and is validated.

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E. MiRNA-15a target identification and binding site analysis using STRING

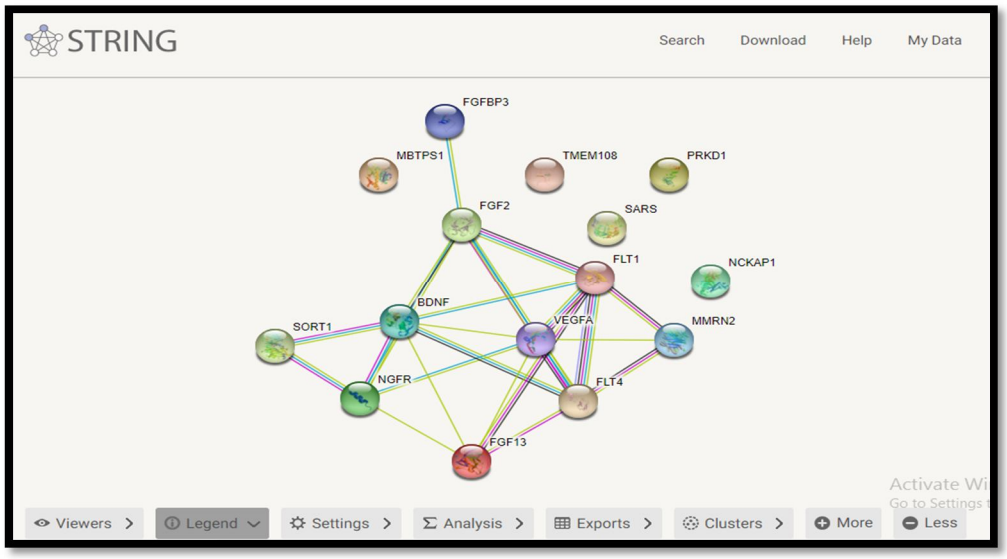


Fig 8: Protein-Protein interaction network visualized by STRING

According to results obtained, FGF2, VEGFA and BDNF shows functional and pathways similarity. Thus, alteration in FGF2 and VEGF will majorly affect the expression of BDNF in vascular endothelial cell through autocrine and paracrine mechanisms which could enhance neoangiogenesis in patients suffering from T2DR.

F. Pathway Enrichment Analysis of miRNA-15a target gene BDNF in T2DR by KEGG

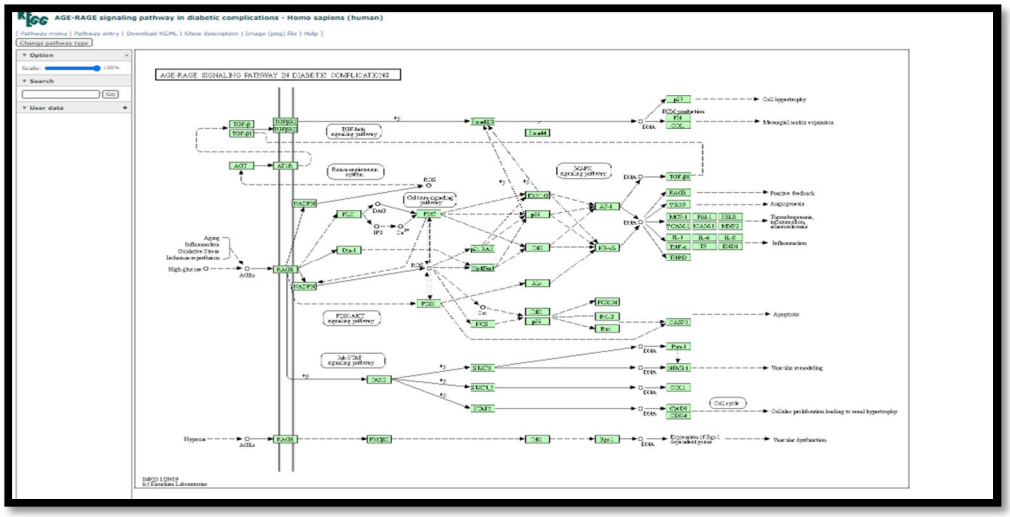


Fig 9: BDNF-MAPK signaling pathway obtained by KEGG for T2DR

BDNF binds to its receptor protein TrkB which undergoes homodimerization, autophosphorylation and activation. It then recruits and activates several downstream effectors to regulate gene expression and cell survival/proliferation. In T2DR patients, due to hyperglycaemia those continuously growing cells will be deprived of oxygen (hypoxia) and BDNF is responsive to hypoxia thus retina will not get nourishment by those cells, due to which retina will send signal to brain for more blood vessels to provide nourishment which leads to neovascularization/neoangiogenesis. Due to BDNF upregulation, highly activation of TrkB receptor expressed on a sub-population of endothelial cells (ECs)/ retinal cells contribute to neoangiogenesis. New vessel formation in T2DR causes visual loss with vitreous hemorrhage, retinal detachment, and neovascular glaucoma which are the complications of T2DR.

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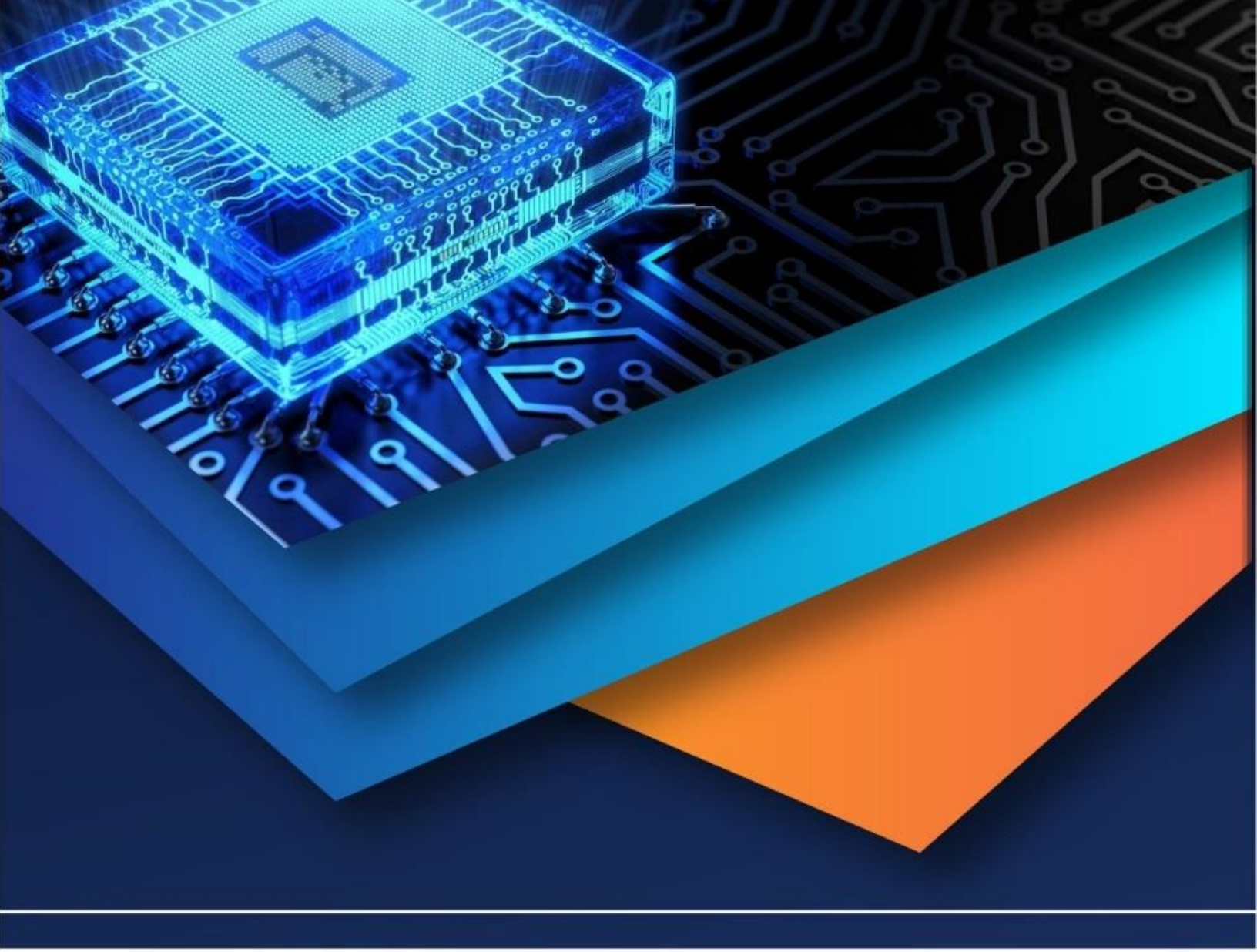
IV. CONCLUSION

Sequence retrieval and functional analysis is first and vital step for the further investigation of a sequence. Hence sequence of has-miR-15a-5p was retrieved using miRBase. It helped in understanding the number of nucleotides present, targeted genes, interaction pattern and functions of miR-15a. Interaction pattern between miR-15a and the neurotrophin, BDNF was also very important for the prediction of changes occurring in the pathways involved to T2DR. From all the analysis conducted using online tools and software and the pathway results studies , it can be concluded that as miR-15a levels declines in diabetic retina or REC, BDNF level may get increase which may further activate downstream effectors and will contribute to neural angiogenesis leading to the development of T2DR. Hence, controlling the miR-15a level will in turn contribute in preventing neoangiogenesis which will help to prevent T2DR.

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