

Review on an in Silico Comparative and Pathway Analysis of Targeted Genes of Diabetic Retinopathy: A Prognostic Approach towards Diabetic Retinopathy

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ABSTRACT:Diabetic retinopathy is the leading cause of visual loss in working-age of adults and is characterized by retinal neurodegeneration and microvascular abnormalities in the eye. DR remains the leading cause of vision loss among adults aged less than 40 years, it was estimated that over 381 million people globally were affected by diabetes, and approximately one-third of them develop some degree of DR. which was classified into non-proliferative DR (NPDR) and proliferative DR (PDR). Without an early screening and effective diagnosis, patients with PDR will develop serious complications. Some studies have sought to identify risk factor for the development of PDR in the early stages; no clinically significant single parameter has been identified. New methods to identify NPDR patients at risk to develop PDR are needed. It is quite essential to explore novel biomarkers with more high sensitivity and specificity for the early detection of PDR for preventing visual loss. Therefore, there is a need for biomarkers which could be used as a tool for the early and specific detection of DR. MicroRNAs are small non-coding RNA molecules which play a key role in controlling gene expression and certain biological processes. Studies show that dysregulation of microRNAs may lead to various diseases including DR, and thus, may be useful biomarkers for disease detection. Therefore, identifying biomarkers like microRNAs as a tool for the early and specific detection of DR, have great potential for diagnostic purposes. The main focus of this investigation, therefore, is the early detection of DR by the identification and validation of novel biomarkers. Furthermore, based on previous studies, the aim of the investigation was to identify differentially expressed miRNAs as well as identify their potential target genes associated with the onset and progression of DR. An in-silico approach was used to identify miRNAs found to be differentially expressed in the serum/plasma of DR individuals. mirwalk which is publically available target prediction software was used for a compressive evaluation of target gene prediction of the identified miRNA. The target genes were subjected to functional analysis using web-based software, namely DAVID. Functions which were clustered with an enrichment score > 1.3 were considered significant. mirwalk was also used for pathway analysis of target gene and its appropriate miRNA.

KEYWORDS:diabetic retinopathy, micro-RNA, novel biomarker.

I. INTRODUCTION

Diabetic retinopathy (DR) is a common microvascular complication of diabetes and the leading cause of legal blindness among people with diabetes [2,38]. Due to the increased lifespan of diabetic patients, the prevalence of DR will continue to increase as a result of the longer duration of diabetes [4,7]. Non-invasive diagnostic methods have been

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Vol. 7, Issue 11, November 2018

shown a lot of interest. Specifically, diagnostic methods which are quick, painless, cost-effective, and sensitive as well as being specific for a particular disease. Most of the current diagnostic tests for most diseases usually involve painful or uncomfortable methods for retrieving samples e.g. tissue samples from biopsies [8,39]. Particular interest is also focussed on diagnostic methods which can detect diseases in its early stages. If diabetic retinopathy were to be diagnosed in its early stages, the development of overt diabetic retinopathy, and its long-term complications, can be avoided. MiRNAs have shown remarkable promise as potential biomarkers for the early (and specific) detection for DR, as well as for other diseases, due to its stability compared to other biomolecules such as mRNA and proteins.

II. DIABETIC RETINOPATHY

Diabetic retinopathy (DR) is a common microvascular complication of diabetes and the leading cause of legal blindness among people with diabetes [2,38]. Due to the increased lifespan of diabetic patients, the prevalence of DR will continue to increase as a result of the longer duration of diabetes [4,7]. Diabetic retinopathy is a most common diabetic eye disease, and also it is a serious eye problem. This disease is caused by diabetes. It affects the retina and causes the blindness in peoples [9,11].The retina is the light-sensitive tissue present at the back of the eye. For good vision a healthy retina is necessary. Diabetic retinopathy is a glucose-induced microvasuclar disease, leading to progressive damage to the tiny blood vessels that nourish the retina [2]. The damaged blood vessels may swell and leak blood and other fluids, causing vision clouding [12].This circulation problems cause retinal tissue to become oxygen deprived, resulting in retinal hypoxia (i.e., lack of oxygen) and irreversible retinal nerve cell degeneration and death. It is a serious sight-threatening complication of diabetes. Diabetic retinopathy usually affects both eyes, leading to significant vision loss if left untreated.

The same scene viewed by a person having Normal vision and with diabetic retinopathy is compared in Figure 1.1-1.2



(a)

(b)

Figure 1.1(a)-1.2(b): (a) scene viewed by a person having normal vision. (b) The same scene viewed by a person with diabetic retinopathy. National_Eye_Institute. [2015, Apr. 21].

All people with diabetes—both type 1 and type 2—are at risk for the development of diabetic retinopathy. The longer the patients have diabetes, the more likely they will develop diabetic retinopathy. Between 40 to 45 percent of Americans diagnosed with diabetes have some stage of diabetic retinopathy [21]. (People with other medical conditions such as high blood pressure and high cholesterol are at greater risk).Pregnant women face a higher risk for developing diabetes and diabetic retinopathy[4]. If gestational diabetes develops, the patients are at much higher risk of developing diabetes as they age. Everyone with diabetes is recommended to get a comprehensive dilated eye examination at least once a year, since macula edema and proliferative diabetic retinopathy can develop without symptoms, such that patients are at high risk for vision loss.

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Vol. 7, Issue 11, November 2018

2.1 Diagnosis and Detection

Diabetic retinopathy and macular edema can be detected through a comprehensive eye examination that includes Visual acuity test, dilated eye exam, and Tonometry. Supplemental testing may include fluorescein angiography [21]. In this test, a special dye is injected into the arm, and pictures are taken as the dye passes through the 12 blood vessels in the retina. The test allows the evaluation and identification of any abnormal blood vessel growth and leaking blood vessels.

2.2 Current Treatments of Diabetic Retinopathy

Treatment of diabetic retinopathy depends on the stage of the disease. During the first three stages of diabetic retinopathy, no treatment is needed, unless macular edema occurs. Laser Surgery, Vitrectomy, Anti - VEGF Therapy and Anti - VEGF Therapy

III. PATHOPHYSIOLOGY OF DIABETIC RETINOPATHY

The pathogenesis of diabetic retinopathy is multifactorial, and a range of hyperglycemia linked pathways was implicated in the development and progression of this condition [2,12]. Initially, retinopathy was characterized solely by microvascular abnormalities, including endothelial cell dysfunction, vessel leakage, and vascular occlusion and degeneration [12]. Recently, there is growing evidence that retinal complications of diabetes are the result of functional and structural alterations in both the microvascular and neuroglial compartments, and DR is widely recognized to be a neuro-vascular disease [3,39]. Such neuronal and glial dysfunction occurs together with blood flow abnormalities and often before the appearance of evident microvascular damage [2,33]. Modifications of astrocytes were observed in a diabetic mice model early in the progression of diabetes. These modifications were accompanied by inner retinal hypoxia and ganglion cell dysfunction. Muller cell gliosis and more extensive changes in neuronal function occurred after the astrocyte alterations [16]. Feng et al. demonstrated that neurodegeneration and glial activation initiated vasoregression in transgenic mice [28]. The exact mechanisms by which hyperglycemia initiated vascular or neuronal alterations in retinopathy have not been completely clarified [12,39]. The cellular damage in the retina was hypothesized to be caused by several mechanisms, including increased flux through the polyol pathway, production of advanced glycation end-products (AGE), increased oxidative stress and activation of the protein kinase C (PKC) pathway [2,9,27]. Nevertheless, human studies or clinical trial are needed to validate many of these hypotheses [2,9,27]. DR has numerous similarities with chronic inflammatory diseases since it is related to increased vascular permeability, edema, inflammatory cell infiltration, tissue destruction, neovascularization, and the expression of pro-inflammatory cytokines and chemokines in the retina. Increased expression of vasoactive factors and cytokines was suggested to play an important role in mediating the structural and functional changes in the retina [20,23]. Recent studies yielded strong evidence suggesting that inflammation was also important in the pathogenesis of early stages of experimental DR. Several inflammatory molecules were shown to contribute to structural or functional alterations that are characteristic of retinopathy [28].

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Vol. 7, Issue 11, November 2018

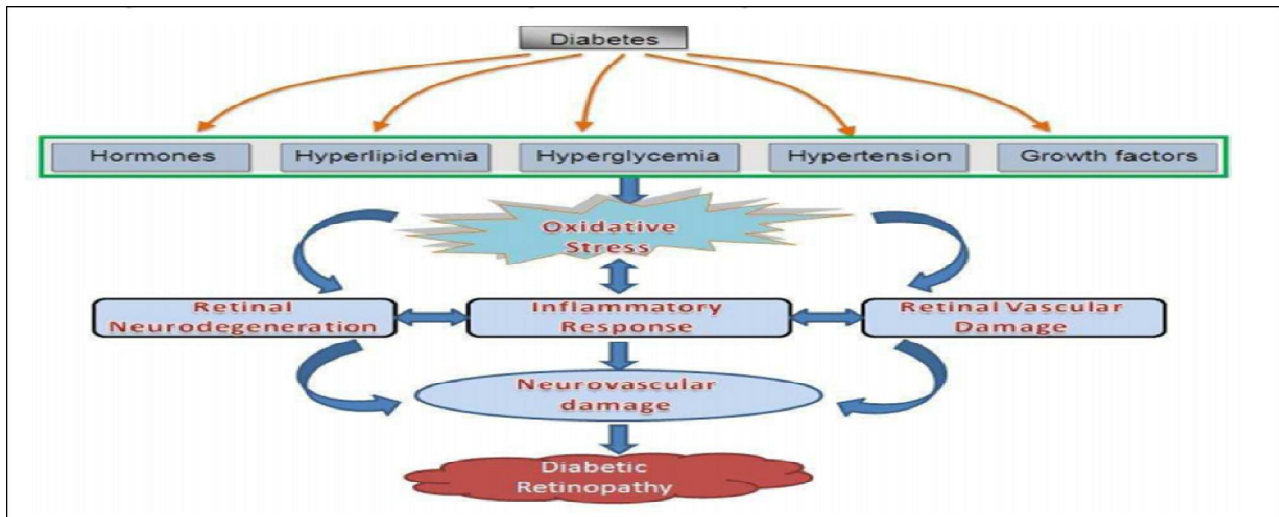


Figure ; 1.3 represented the pathophysiology of DR <http://dmm.biologists.org/content/5/4/444>

IV. LIMITATION OF DIAGNOSIS

Overall, the pathogenesis of DR is multifactorial, with a complex interplay of microvascular, neurodegenerative, genetic/epigenetic, immunological, and secondary inflammation-related factors. DR remains the leading cause of vision loss among people with diabetes it was estimated that over 381 million people globally were affected by diabetes, and approximately one-third of them develop some degree of DR. Current treatment modalities for DR, such as laser photocoagulation, intravitreal corticosteroids, intravitrealantivascular endothelial growth factor (VEGF) agents, and vitreo-retinal surgery, are applicable only at advanced stages of the disease, have large patient-to-patient variation, and are associated with significant adverse effects.[5,35].Therefore, more systems level understanding of the underlying molecular pathomechanisms, novel prognostic indicators, and pharmacological treatment options of the different stages of the DR are required.

V. THE NEED FOR NOVEL BIOMARKERS FOR THE DETECTION OF DISEASES

Based on the above-mentioned limitations, there is a need for novel biomarkers which can detect the disease at earlier stage with effective way possibly which could act as a supplementary test to current diagnostic methods. Biological molecules serve as biomarker (e.g. genes, proteins, and metabolites) in biological fluids (e.g. serum, urine, saliva) or tissue/cells, it can aid in indicating normal or abnormal processes in the body, or a specific disease condition [44]. Furthermore, they are also important for the diseases detection at earlier stage [44]. The process of discovery of biomarkers is a lengthy and challenging. Good biomarkers have to be sensitive, specific, and it must be standardised and reproducible [44]. Many strategies have been used over the years for the identification of new biomarkers [11,44]. Computational biology (or bioinformatics) has played an instrumental role in the discovery of new biomarkers as well as the validation of potential biomarkers [11,44].

VI. BIOGENESIS OF MIRNAS AND THEIR USE AS BIOMARKERS FOR DISEASES

Biogenesis of miRNAs is tightly regulated at the transcriptional and posttranscriptional levels, as shown in Figure. MiRNAs are encoded in the genome and undergo transcription in a similar process to protein coding genes i.e. by both RNA polymerase II or RNA polymerase III [30,40], forming a stem-looped molecule known as the primary miRNA (pri-miRNA) transcript within the nucleus. The pri-miRNA is then cleaved by Drosha (an RNase III enzyme) and the

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Vol. 7, Issue 11, November 2018

DGCR8/Pasha protein complex, resulting in a ~70 nucleotide hairpin miRNA precursor (pre-miRNA) structure [6,11,18], which is transported into the cytoplasm by Exportin-5 [6,11,23]. The pre-miRNA is cleaved a second time by another RNase III enzyme, Dicer, to yield a miRNA duplex structure [18,30] approximately 22 nucleotides in length [18]. The duplex then separates into two single miRNA strands which binds to an Argonaute-2 (Ago2) protein and is incorporated into the RNA-induced silencing complex (RISC) structure; this helps the miRNA to identify and bind to the complementary sites of its target mRNA [18] where it will inhibit gene expression.

Primary miRNA transcripts (pri-miRNA) transcribed by RNA Polymerase (pol II or III) and processed by microprocessor (Drosha and DGCR8), are exported as pre-miRNAs into the cytoplasm by Exportin-5 and RanGTP. Dicer and its interacting binding protein TRBP process pre-miRNAs into short miRNA duplexes. The 5' mature miRNA strand binds to the RNA-induced silencing complex (miRISC)-associated argonaut protein (Ago2), and induces silencing of their mRNA target sequences. The regulation of miRNAs has been linked to many important physiological and pathological processes [17]. Therefore, miRNAs have shown promise as ideal tools for diagnostic, prognostic, or monitoring therapeutic success of diseases [17,34]. The advantage of using miRNAs over other biological markers (e.g. proteins or mRNA), is that they are stable and can be expressed in degraded RNA samples from human tissue [17,19]. Furthermore, circulatory miRNAs, which have been detected in body fluids such as blood, urine and saliva [20], Circulatory miRNAs is of particular interest for highly-sensitive non-invasive diagnostic purposes for the early detection of many diseases [1,17]. As an intriguing and potent mediator in the pathological mechanisms of diabetic retinopathy, microRNA (miRNA) have been investigated for the expression profile and regulatory networks during the last decade the level of miRNA expression differs between different types of cells and disease states. miRNA have been implicated as a promising candidate of molecular therapeutics to protect damaged and injured retina, as they have the capacity for gene regulation and can be biomarkers for many human diseases. The relevance of miRNA on the pathological mechanisms of diabetic retinopathy was first shown approximately a decade ago. We still have little knowledge which miRNA are involved in the onset and progression of diabetic retinopathy. A small number of studies have identified the expression and functions of specific miRNA in diabetic retinopathy [22,25,29]. Computational analyses have been done to find regulatory networks of miRNA, but the majority of miRNA targets have not been experimentally tested. Moreover, little has been shown for the regulatory networks of miRNA in diabetic retinopathy.

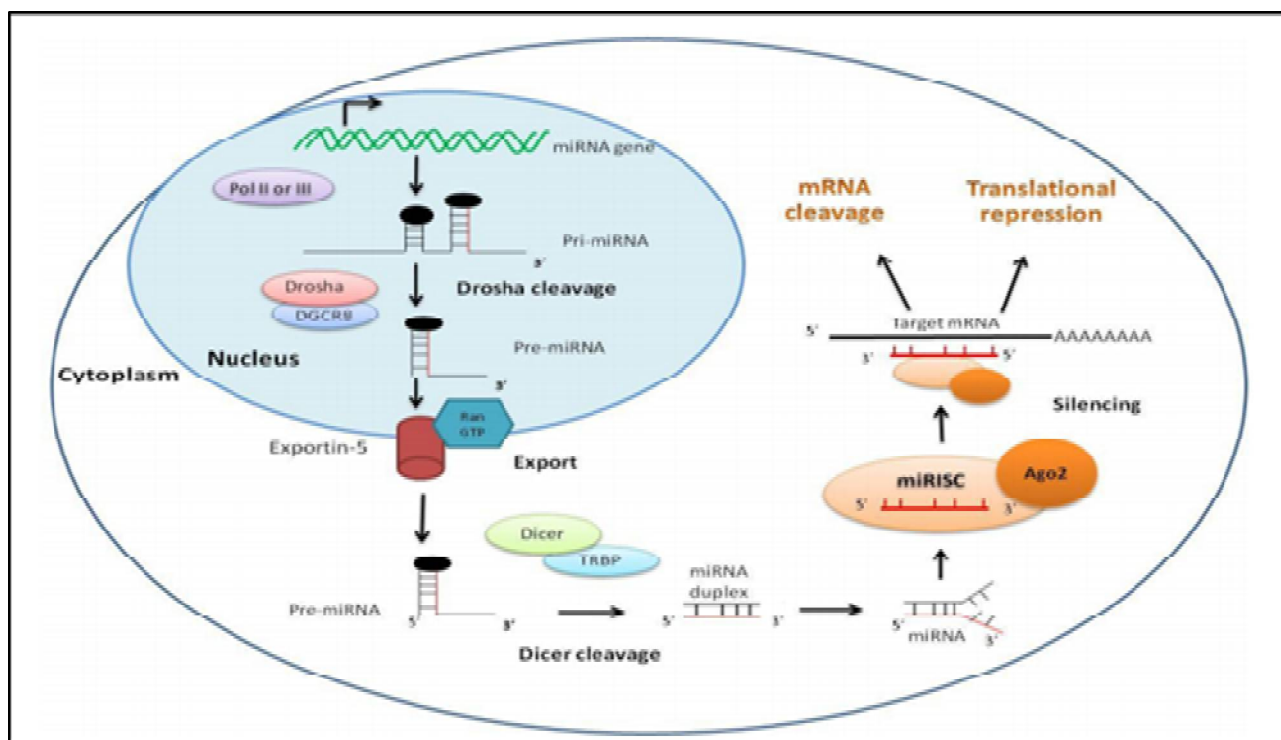


Figure 1.4 Schematic diagram representing the biogenesis of miRNA and its mechanism of action. (<https://spchanlab.wordpress.com/microrna-biogenesis-and-function/>)

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Vol. 7, Issue 11, November 2018

VII. BIOINFORMATICS AS A TOOL FOR THE DETECTION OF NOVEL BIOMARKERS

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 purposes[31]. Additionally, bioinformatics allows for in silico simulations of complex disease physiologies, such as interactions between components, on their molecular level[11]. Role of bioinformatics in miRNA research Identifying miRNAs, their target genes, and their respective regulatory function are important for understanding normal biological processes as well as understanding their various roles in disease development[17,26,45]. Potential target identification is based on the software's algorithm. There is several miRNA-target prediction software that is publicly available [17] which include TargetScan, PITA and Diana Micro-T[41]. Possible targets are predicted based on the 29 software's prediction algorithm, which is categorized into three groups: (i) sequence-based, (ii) energy-based, and (iii) machine learning-based [45].

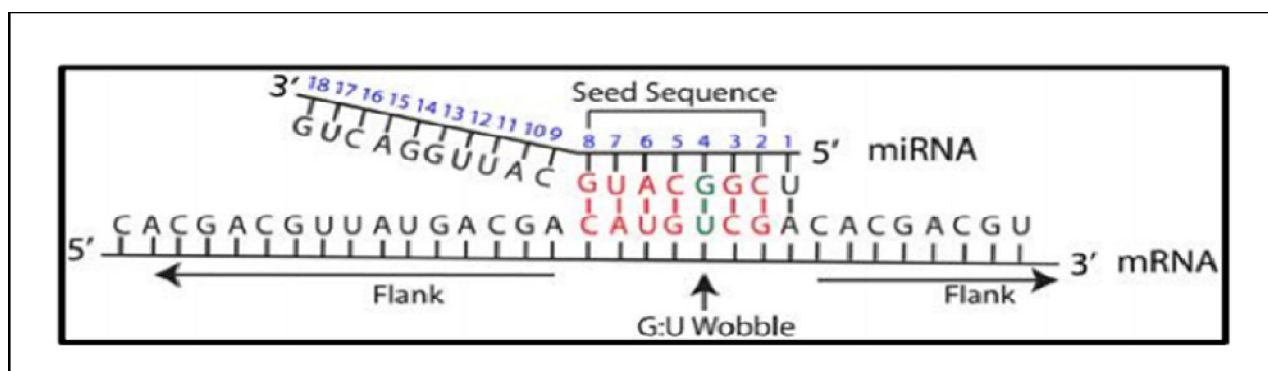


Figure 1.5 Schematic overview of the miRNA:mRNA target interaction.

Watson-crick base pairing of the miRNA seed sequence and the mRNA target sequence is shown in red, and an example of a G-U wobble in the seed sequence is shown in green. Flank refers to the 5' or 3' mRNA sequence corresponding to the region on either side of the seed sequence (Adapted from: Peterson et al., 2014).

VIII. CONCLUSIONS

There is a need for identification and validation of good biomarkers Particular interest is also focussed on diagnostic methods which can detect diseases in its early stages. If diabetic retinopathy were to be diagnosed in its early stages, the development of overt diabetic retinopathy, and its long-term complications, can be avoided. MiRNAs have shown remarkable promise as potential biomarkers for the early (and specific) detection for DR, as well as for other diseases, due to its stability compared to other biomolecules such as mRNA and proteins.

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