

Epigenetic Modification of 5'methylcytosine DNA Methylation in the CpG Island of promoter region of Brain Derived Neurotrophic Factor for the pathogenesis of Type 2 Diabetic Retinopathy

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Abstract: Type 2 Diabetic Retinopathy is one of the leading causes of blindness world wide .It remains one of the most devastating chronic complications and is caused by changes in the blood vessels of the retina. Type 2 Diabetic Retinopathy (T2DR) is broadly recognized as a neurovascular disease. Patients suffering from T2DR are one third of human population suffering from diabetes mellitus. Retina is a neuronal tissue of the eye produces neurotrophic factors for its maintenance. Among neurotrophin, Brain derived neurotrophic factor (BDNF) mainly abundant in blood serum supplies over retina through microvascular system and effective in protecting retina from hyperglycemia. BDNF helps retinal cell survival in a concentration dependent Manner. BDNF may protect retina from hyperglycemia via the TrkB/MAPK pathway and provides pathogenesis of T2DR.DNA methyltransferases (Dnmts) catalyses methylation of cytosine to 5-methyl cytosine (5mC).Dnmts a family with five different members—Dnmt1, Dnmt2, Dnmt3a, Dnmt3b and Dnmt3L; out of which only Dnmt1 Dnmt3a and Dnmt3b are catalytically active. Over expression of Dnmt1 leads to down regulation of BDNF gene which causes apoptosis of retinal cells. It has been observed that level of BDNF dysregulates during the pathogenesis in T2DR patients. Present review concluded that the level of 5' methyl cytosine DNA methylation on BDNF in a retinal cell affects the microvasculature of human retina that can named as neovascularization or any retinal microvasculopathy. The Expression level of 5'methyl cytosine DNA methylation on BDNF between the patient and a healthy person can be important in the establishment of cause of T2DR and may provide hints about their unique role in the pathogenesis of disease.

Keywords: Type 2 Diabetic Retinopathy, Epigenetic, DNA Methylation, Brain derived neurotrophic factor, DNA methyltransferase.

I. INTRODUCTION

Type 2 Diabetic Retinopathy is the most frequently occurring microvascular complication of diabetes and, although not all patients will suffer significant vision loss, this condition remains a leading cause of blindness. Moreover, ~80% of insulin-dependent Type 2 diabetic patients and 50% of Type 2 diabetic patients not requiring exogenous insulin will contain retinopathy after 20 years of diabetes [1,2] . Diabetes and diabetogenic agents such as high glucose, Ages, TGF- β undesirable effects in major target cells involved in vascular dysfunction, including endothelial cells and retinal cell [3-11]. There is retinal cell apoptosis and angiogenesis in case of T2DR.Hyperglycemia is considered as the leading cause of the development of diabetic retinopathy [12-

14]. A number of metabolic abnormalities initiated by hyperglycemia are implicated in the growth of T2DR.DNA methylation is one of the epigenetic mechanism involving the transfer of a methyl group onto the C5 position of the cytosine to form 5-methylcytosine. DNA methylation regulates organic phenomenon i.e gene expression by recruiting proteins which are involved in gene repression or by inhibiting the binding of transcription factor(s) to DNA [15]. Retinal Endothelial cell under the conditions of hyperglycemia were used as a model to study apoptosis in diabetic retinas in case of T2DR. Retinal Endothelial cell exposed to hyperglycemia exhibited high levels of apoptosis. Brain derived neurotrophic factor (BDNF) is one of the member of the neurotrophin family which is helpful in protecting retinal cell from hyperglycemia.

From this study we may analyse the status of 5'methylcytosine DNA methylation level in the CpG Island of promoter region of bdnf gene through TrkB / MAPK signalling pathway in case of T2DR.

II. EPIGENETICS AND GENE REGULATION

Epigenetics is now rising as one of the important factors in T2DR diseases as it can control the complex interplay between genes and the environment, and these heritable changes can occur without any change in the DNA sequence. Epigenetic change is considered to be a regular and natural phenomenon which can be influenced by several factors including the age, the environment, lifestyle and the state of the disease [16,17]. Epigenetic modifications acts like switches which helps in managing gene activity and allowing alternations in genome function without altering the gene sequences. DNA methylation, Histone modification and noncoding RNA are considered to initiate and sustain changes in gene regulation which are the three major epigenetic modifications [18-20].

III. TYPE 2 DIABETIC RETINOPATHY

T2DR is taken into account to be one in all the foremost feared microvascular complications of polygenic disease, and hyperglycemia remains because the major instigator of its development. Changes in the blood vessels of the retina can cause T2DR. When these blood vessels area get injured, they leak blood and grow

fragile new vessels. Once the nerve cells area gets damaged, vision is impaired. Diabetic retinopathy can cause vision loss in two different ways: a) Macula Edema, b) Proliferative retinopathy.

Type 2 Diabetic Retinopathy classified into two clinical forms they are: non proliferative diabetic retinopathy, proliferative diabetic retinopathy. Non proliferative diabetic retinopathy subdivided into 3 different stages they are mild, moderate and severe [21, 22]. Non proliferative diabetic retinopathy is being characterised by the quantity of microaneurysms, dot and blot haemorrhages, cotton wool spots and blood vessel abnormalities [23]. Proliferative diabetic retinopathy is the advanced stage, which involves the development of neovascularisation which develops from venous side of the retinal circulation and may penetrate the inner limiting membrane into the vitreous [21,22]. In case of proliferative diabetic retinopathy improvement of glaucoma, retinal detachment, and vision loss may additionally manifest [24].

Diabetic maculopathy is the process in which Type 2 Diabetic Retinopathy affects the macula and central vision acuity. Diabetic macular oedema occurs when the area of retina, excessive vasopermeability, oedematous were damaged. The most common cause of blindness in diabetic is diabetic macular oedema [25].

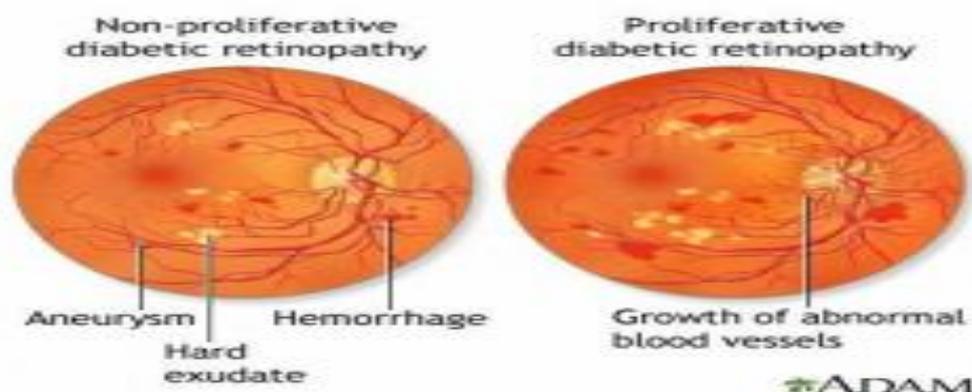


Fig-1: **Types of Diabetic Retinopathy** (<https://www.pinterest.com/pin/377317275008848200/>)

T2DR is being considered as one of the microvascular complication of retinal endothelial dysfunction, as it is characterized by pericyte and endothelial cell loss, capillary basement membrane (BM) thickening, blood-retinal barrier (BRB) breakdown and leakage, a cellular capillaries, and neovascularization where all types of retinal cells are affected including ganglion cells [26-28]. In case of T2DR, reduction in synapse numbers and synaptic proteins, thinning of the inner nuclear layer (INL), alteration in dendrite morphology, and retinal pigment epithelium (RPE) dysfunction occurs which result in the

gradual loss of retinal cell function [28]. In early stage of T2DR, glia activation and innate immunity/sterile inflammation [26, 27] occur. Therefore, T2DR is not only a vascular disease but also a neurodegenerative disease.

In the retina, oxidative stress increases due to hyperglycemia, and the enzymes which are responsible for DNA methylation are modified by reactive oxygen species (ROS) [29]. The expression of targeted genes is altered due to epigenetic modification in various genes

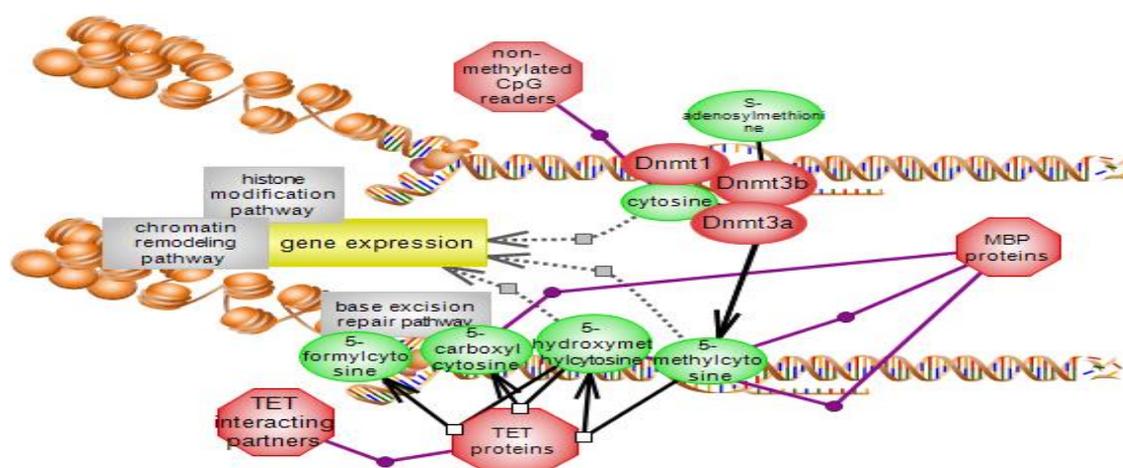
and transcription factors which result in mitochondria dysfunction and ultimately leads to T2DR.

IV. DNA METHYLATION AND ITS FUNCTION AND MECHANISM

DNA is not an unchanging entity but instead it is highly dynamic and it responds to the environmental stimuli by modifying its properties in adapting to the changes [30]. Methylation of cytosine to five-methyl cytosine (5mc) is considered as one of the predominant epigenetic modification; methylation of the CpG islands, a CG rich location within the promoter of many genes, changes Protein-DNA interactions leading to changes in chromatin structure, and this hampers the binding of transcriptional equipment, ensuing in gene suppression [31,32]. DNA methylation is catalyzed through DNA methyltransferases (Dnmts), a family with five distinct participants—Dnmt1, Dnmt2, Dnmt3a, Dnmt3b and Dnmt3l; out of which only dnmt1, Dnmt3a and Dnmt3b are catalytically energetic. Dnmt3a and Dnmt3b are de novo methyltransferases, and dnmt1 is a maintenance enzyme

Dnmt1 is important in regulating tissue-specific patterns of methylated cytosine residues [33, 34]. Pathological Dnmts activity and abnormal 5mC formation have been linked with neurodegeneration [35]. Subfamily of DNA glycosylases has been considered to promote active DNA demethylation by removing the 5-methylcytosine base, followed by DNA backbone cleavage at the abasic site, and the cytosine which is methylated is replaced by an unmethylated cytosine [36]. The passive process results in hypo methylated DNA in the absence/inactivation of Dnmt1 [37]. In order for the DNA methylation pattern to be changed, there must be both active DNA methylation and demethylation in the retinal genome [38].

The CpG sites or CG sites are regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases along its 5' → 3' direction. CpG is shorthand for 5'-C-phosphate-G-3', that is, cytosine and guanine separated by only one phosphate; phosphate links any two nucleosides together in DNA.



ELSEVIER

Fig-2: DNA methylation modification pathway

(http://rgd.mcw.edu/rgdweb/pathway/pathwayRecord.html?acc_id=PW:0001340)

In case of T2DR, Dnmts enzyme activity is increased in the retina and its capillary cells and the expression of Dnmt1 (protein and gene), but not of Dnmt3a or 3b, is also remarkably elevated [39]. Hence proved that Dnmt1 continues to be over expressed even after termination of hyperglycemia, signifying its role in the metabolic memory phenomenon. Consistent with results, differential DNA methylation patterns are observed in the blood samples of patients with proliferative diabetic retinopathy [40].

Mitochondria are considered to play an important role in the development of T2DR, and their

homeostasis is disturbed, and even after termination of hyperglycemic insult they remain dysfunctional [41-44]. Dnmt1 has a mitochondrial targeting sequence, which helps its transport into the mitochondria [45], and in case of diabetes, Dnmt1 is elevated inside the retinal mitochondria and thus mtDNA is hypermethylated, which may leads to T2DR [39]. It has been reviewed that reversal of hyperglycemic insult does not benefit hyper methylation of mtDNA; the binding of Dnmt1 and 5mC levels at the regulatory region of mtDNA, the area that is more prone to damage, remains promoted for a period of good glucose exposure as hyper

methylation is associated with transcription inactivation[46,47].

V. BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF)

Brain-derived neurotrophic factor (BDNF) is one of the members of the neurotrophin family which has an important role in neural cell differentiation, nerve cells survival, neurite outgrowth, and synaptic plasticity. BDNF control the development, plasticity and survival of cholinergic, dopaminergic, and serotonergic neurons. It also controls glutamatergic neurotransmitter which release and promotes the development of GABAergic neurons. In the mammalian brain, including the cerebral cortex, basal forebrain, hypothalamus, hippocampus, striatum, limbic structures, brainstem, and cerebellum BDNF is largely expressed [48].

Human BDNF consists of complex gene structure of 11 exons (I–V, Vh, VI–VIII, VIIIh, IX), 9 of which (exon I–VII, IX) contain functional promoters. Exons II, III, IV, V, Vh, VI, and VIIIh do not have a translation start site so translation of these exons starts from the ATG of exon IX. [49].The use of translation start sites in exons I, VII, and VIII of BDNF can lead to the pre-proBDNF proteins with longer N-termini. And different splice sites are situated in exons II, V, VI, IX.

Exon IX contains two different polyadenylation sites, use of different splice sites leads to the formation of numerous BDNF transcripts variants that determine a tissue-specific BDNF expression regulation as well as the regulation in responses to environmental stimuli and signaling events [50, 51]. BDNF gene most abundant in retina any Dysregulation in the level of BDNF may leads T2DR.

Genomic View for BDNF Gene

Genes around BDNF on UCSC Golden Path with GeneCards custom track

Cytogenetic band: 11p14.1 by Ensembl 11p13 by Entrez Gene 11p14.1 by HGNC

BDNF Gene in genomic location: bands according to Ensembl, locations according to GeneLoc (and/or Entrez Gene and/or Ensembl if different)

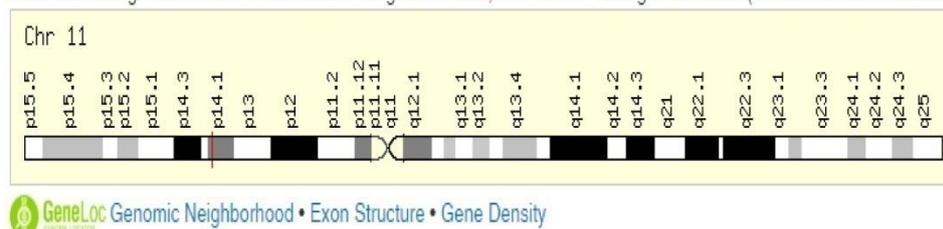


Fig-3: Structure of BDNF gene

BDNF has a high affinity for TrkB and p75 enhances the interaction between BDNF and TrkB [52].TrkB homodimerization, autophosphorylation and activation. It after that recruits and activates several downstream effectors to regulate gene expression and protect retinal cell. Members of the TrkB downstream signalling cascade, including PI3K/PKB and ERK/MAPK, have been observed to be responsive to BDNF [53, 54].Apoptosis which is also known as programmed cell death is important in multicellular organisms. Numerous proteins regulate apoptosis and genes like BDNF which activates the TrkB-ERK/MAPK pathway and inhibits apoptosis by regulating the 5'methyl cytosine DNA methylation level which in turn regulate BDNF and thus promoting the survival of retinal cell from hyperglycemia and provides novel insights into the pathogenesis of Type2 Diabetic Retinopathy.

In the animal model of T2DR, few studies showed that reduced level of BDNF in the T2DR may damage neurons, thereby leading to neurodegeneration

[55]. Another study reported that neurotrophic factors may induce neoangiogenesis through endothelial progenitor cells activation, leading to the pathological retinal neovascularisation [56] found that BDNF was effective in protecting retinal neurons from hyperglycemia in vitro. The patients with T2DR were found to have a drastic reduction in the level of BDNF in retina [57].

In this pathway if any alteration occurs in any signalling molecule such as BDNF, which in turn alter TrkB (receptor of BDNF) it may lead to cause Type 2 diabetic retinopathy.

BDNF levels are lower in individuals with T2DM compared to non-diabetic individuals, both in plasma and serum, and in different populations [58, 59]. It has been observed that difference in serum BDNF levels, DNA methylation patterns and genetic variants, across different glucose metabolic state advice that BDNF may be involved in the pathophysiological

process of insulin resistance and Type 2 Diabetes which may cause T2DR.

VI. CONCLUSION

Epigenetic mechanisms are hypothesized to play an important role in the case of Type 2 Diabetic Retinopathy because they provide a mechanism for continued altered gene expression. It has been reported that hyperglycemia can induce site-specific DNA methylation.

The available literature reviewed thoroughly concluded that level of DNA methylation in BDNF in retinal cells may affect the microvasculature of human retina that can be named as neovascularization or any retinal microvasculopathy. The Expression level of DNA methylation of BDNF gene between the patient and a healthy person can be important in the establishment of cause of Type 2 Diabetic Retinopathy and hints about their unique role in the pathogenesis of disease.

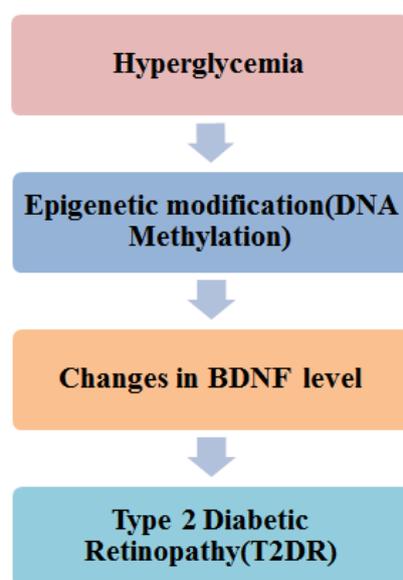


Fig-4: Flow chart of T2DR

Hyperglycemia, a condition where glucose level increases leads to epigenetic modification like DNA methylation which then alters BDNF gene expression and due to that BDNF protein level changes which may lead to T2DR.

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