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Epigenetics changes of Brain Derived Neurotrophic Factor in case of Hypermethylation in Type 2 Diabetic Retinopathy

Anuradha Agrawal[1], Akash Zararia[2], Priyanka Mishra[3], Archana Tiwari[4]
[1, 2]Students, [3]Assistant Professor, [4]Head of the Department, School of Biotechnology, Rajiv Gandhi Proudyogiki Vishwavidyalaya (State Technological University), Bhopal, Madhya Pradesh-462033, India

Abstract: ***Recently visual impairment in adults due to hyperglycemia is primarily attributed to Type 2 Diabetic Retinopathy. Type 2 Diabetic Retinopathy is the end result of hyperglycemia which leads to separation of the retina causing blindness. Epigenetic alteration in disease development is attributed to the interaction between genetic and environmental factors. For the pathogenesis of Type 2 Diabetic retinopathy epigenetic changes can play a major role.***
Recent studies shows that through the TrkB/ERK/MAPK pathway BDNF can protect retinal neurons from hyperglycemia and also provides novel insights into the pathogenesis of Type 2 Diabetic Retinopathy.
It is formulated that increased level of glucose may inhibit the conversion of DNA methylation (5mC) to hydroxymethylation (5hmC) by the ten-eleven translocation (TET) dioxygenases family of proteins by influencing α-ketoglutarate levels. Under this hypothesis, increased level of blood glucose would increase 5hmC levels due to elevated TET activity, which in turn would deregulate gene expression. The main outlook of this review is the regulation of hypermethylation by epigenetic changes of TET protein-mediated in DNA methylation/ demethylation in BDNF for a detailed insight into the pathogenesis of Type 2 Diabetic retinopathy.
Keywords: brain derived neurotrophic factor, epigenetics, Ten- eleven translocation protein, 5hydroxymethylcytosine, ERK/MAPK signaling pathway

I. TYPE 2 DIABETIC RETINOPATHY

Type 2 Diabetic Retinopathy is considered the primary cause of visual impairment in 90% of adults with more than 20 years of diabetes. Due to this disease our society carries a heavy burden as it is responsible for 4.8% of the 0.037 billion cases of eye disease related blindness all over the world. As the time is passing diabetes is increasing at an alarming rate. The number of people with diabetic retinopathy is expected to increase from 0.1266 billion in 2010 to 0.191 billion by 2030 [1].

This ailment is specially signalized by damage of the microvasculature of the retina. There is some early histopathological changes of this slowly processing disease are: microaneurysms, hemorrhages, intraretinal microvascular abnormalities, cotton wool spots and venous bleeding.

But, in higher stages, new delicate vessels are framed alongside the retina and on the back surface of the vitreous, and if not treated on time, they result in the separation of the retina, prompting visual deficiency [2]. Even though hyperglycemia is the main reason behind Type 2 Diabetic Retinopathy, hypertension and dyslipidemia also considered considerable risk factors in the development and progression of T2DR [3–5].

Regardless of broad research related to the pathogenesis of diabetic retinopathy, the precise mechanism remains mystifying making it a daunting task to inhibit progression of this disease. Number of probable mechanisms include in its development like oxidative stress, increased formation of advanced glycation end products, and activation of protein kinase-C, polyol production, and hexosamine pathways [2, 6, 7]. These pathways are interconnected [8], further complicating strategies to prevent the development/progression of this devastating diabetic complication.

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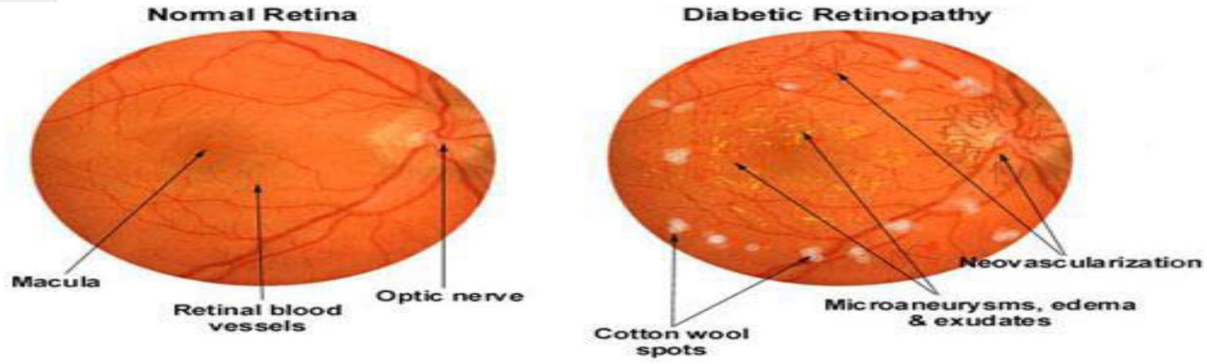

Figure 1: Normal Retina Image and Diabetic Retinopathy Retina Image [9, 10].

Epigenetic modification involves the interaction of genetic and environmental factors that lead to the development of disease. This review focuses on the epigenetic changes in diabetic retinopathy and their methylation of the 5HMC region with the aid of TET protein and the 5HMC might treat the Type 2 Diabetic Retinopathy in the *BDNF* gene [11]. This review defines the epigenetic changes in the *BDNF* gene at the specific region at 5hmc in Type 2 Diabetic retinopathy. This literature may be useful for diagnosing and also for therapeutic purpose because this review shows the relation between the *BDNF* and the Type 2 Diabetic retinopathy, *BDNF* mainly present in the brain and eyes. This study all about the epigenetic modification in the *BDNF* gene specifies the 5hmc region.

II. BDNF AND TYPE 2 DIABETIC RETINOPATHY:

BDNF (brain-derived neurotrophic factor) is a protein which is member of the neurotrophin family of growth factor and it is encoded by *BDNF* gene and it is essential in the development, differentiation, and maintenance of neurons. BDNF gene of humans has a complex structure, that consists of 11 exons (I–V, Vh, VI-VIII, VIIIh, IX), of which 9 exons have functional promoters (exon I–VII, IX) and the exons which don't have translation start sites(i.e. II, III, IV, V, Vh, VI, and VIIIh) so their translation starts from the ATG of exon IX [91]. Prior studies showed that *BDNF* is important for optic nerve, repair of damaged retina and the photoreceptor cells. *BDNF* encourages survival in injured retinal ganglion cells induced by axotomy or retinal ischemia and also promotes nerve fiber regeneration [12, 13]. In addition, *BDNF* helps the survival of retinal interneuron's and is essential for synaptic connections in the developing retina and establishing phenotypes [14]. In past studies it's been reported that *BDNF* inhibit neuroretinal cell death in the case of ischemia and hypoxia to inhibit apoptosis in rat retinal ganglion cells (RGCs) at early stages of DR [15]. However, the mechanisms by which *BDNF* regulates RGCs still remain unclear [16-18].

Tropomyosin-related kinase B (TrkB) is a receptor protein that participates in the central and peripheral nervous systems 'maturation and development. *BDNF* has a high affinity to TrkB and p75 improves TrkB-*BDNF* interaction [19]. TrkB undergoes the process of homodimerization and autophosphorylation, and then activation after ligand binding. It then interacts and activates various downstream effectors for expression of gene regulation and neuronal protection. It was reported that members of the downstream signaling cascade of TrkB, including PI3K/PKB and ERK/MAPK, responded to *BDNF* [20, 21]. A number of studies assumed that the ERK/MAPK pathway mainly activates by *BDNF* [22-25]. Other studies, on the other hand, have reported that cell death occurs due to activation of the ERK/MAPK pathway and PI3K/PKB is the main pathway involved in the *BDNF*-induced neuron protection [26]. Furthermore, both pathways have been reported to be critical for *BDNF*-induced neuroprotection. According to the precvious studies there are no such evidence that addresses the pathways that influence the TrkB activation response [21, 27, 28].

Several studies in vivo have confirmed that the death of retinal neurons in earlier stages of DR correlates with lower levels of *BDNF* and that TrkB is needed to protect *BDNF*-induced retinal ganglion cells and brain neurons [29].

Liu and his colleagues in their in vitro studies demonstrate that Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, helps to protect neurons of retina from hyperglycemia. BDNF promoted concentration-dependent survival of neuronal cells. In addition, BDNF has been shown to promote tropomyosin-related kinase B (TrkB) expression and elevate TrkB and ERK phosphorylation levels in the neurons of retina which are exposed to hyperglycemic condition. Their study results showed that BDNF can defend retinal neurons from hyperglycemia through the TrkB/ERK/MAPK pathway and provides new understanding into DR pathogenesis [29].

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III. EPIGENETICS

In 1942, Conard Waddington gave the term epigenetic as a concept of environmental effect when it came to modifying the phenotype [30]. Epigenetics is the study of mitotically heritable changes in gene expression potential that are not mediated by changes in DNA sequence while genetics is the study of heritable changes in gene activity or function due to the direct changes of the DNA sequence.

Such alterations include point mutations, deletions, insertions, and translocation. In comparison, epigenetics is the study of heritable changes in gene activity or function that is not related to any change in the DNA sequence itself [31]. Epigenetic modifications, as well as methylation, acetylation, phosphorylation, and ubiquitination, alter the interaction between the DNA, histones and nuclear proteins, thus affecting transcription of genes and regulating silencing or expression of genes [32]. In several cellular processes, epigenetic mechanisms have established them as key players, including cell differentiation, DNA replication repair and aging [33-36].

These epigenetic mechanisms play a key role in the developmental origins of health and disease [37]. Epigenetic regulation is critical for mammalian development and cellular differentiation, and small changes in the epigenome (epigenetic dysregulation) causes a wide range of adult-onset chronic diseases [38].

Although there is a uniform definition of epigenetics, it is described as heritable variations in gene function that occur without changing the nucleotide sequence [39]. Epigenetic mechanisms include DNA methylation/de-methylation, noncoding RNA activity and histone modifications to regulate gene expression [40-43]. Epigenetic mechanisms highly associated with T2DR, this review focus on Epigenetic Modification of TET mediated DNA methylation/de-methylation in Brain-Derived Neurotrophic Factor for the Pathogenesis of Type 2 Diabetes Retinopathy.

Epigenetic changes in Diabetic retinopathy: It has become clear that diabetic background interrupts metabolic homeostasis and also modifies different genes, including genes associated with oxidative stress, inflammation and apoptosis [44-47], both genetic susceptibility and environmental factors play critical roles in the development of metabolic diseases including obesity and DR. Epigenetics refers to alterations in gene expression that are unrelated to changes in the DNA sequence that can be inherited and affected by environmental factors [48]. Changes in genes can also be controlled to control gene expression by transcriptional and translational initiation without altering the nucleotide composition of the genome [39]. Recent studies have shown that in many chronic diseases such as diabetes and its complication, epigenetic alterations play an important role.

IV. DNA METHYLATION AND DNA DE-METHYLATION

DNA methylation, is major epigenetic mechanism involving addition of a methyl group using enzyme S-adenosyl methionine (SAM) as methyl donor at position 5 of cytosine residues of the CpG dinucleotide cluster(CpG Island), the regulatory region of most genes, is typically associated with transcriptional repression [49, 50].

The process of methylation involves the unintended changes brought about by the environmental factors or other lifestyles, and these changes could be passed on for several generations. 5-methylcytosine (5mC) generated by Cytosine 5 methylation and this reaction is catalyzed by DNA (cytosine-5) methyltransferases (Dnmts).

Then, Ten-eleven-translocation enzymes (TETs) facilitate 5-methylacytosine (5mC) to 5-hydroxymethylcytosine (5hmC) [51, 52], making these enzymes can be expected target for pharmacological regulation. Although, how the process of methylation-demethylation of DNA remained uniformed is still unclear (53).

DNMTs enzymes catalyze the cytosine methylation by transferring of methyl group to the position C5 in the process of DNA methylation and de-methylation pathway.

TET enzymes can catalyze the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine. In 2009, further help of TET enzymes, two more cytosine analogs have been discovered, TET-dependent oxidative reactions lead to a conversion of 5hmC into 5fC and 5caC. 5fC and 5caC are recognized and removed by TDG, repaired by the base excision repair pathway generating an unmodified cytosine.

During DNA replication dilution of modified cytosine's, 5mC or 5 hmC, can also yield unmodified cytosine, through a mechanism termed passive DNA de-methylation, which is either TET-independent or TET-assisted, respectively. Replication-coupled dilution of 5fC and 5caC by passive DNA de-methylation are not depicted [54].

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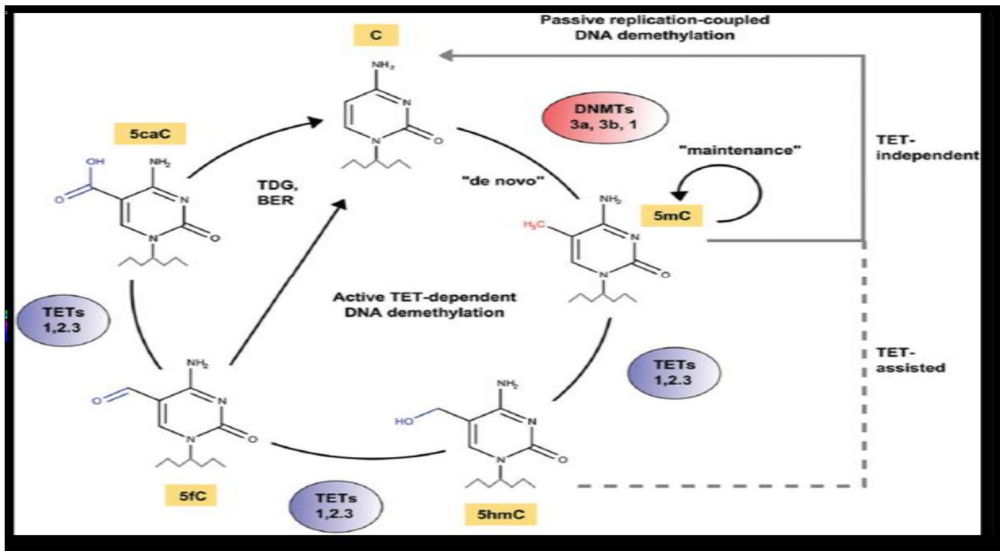

Figure2. DNA methylation pathway [63]

DNA methylation considered as one of the most significant epigenetic changes. DNA methylation of the CpG islands, a CG rich region in the promoter of many genes, changes protein-DNA interactions that lead to changes in structure of chromatin, and this affects the binding of transcription mechanism, resulting in gene suppression [55, 56]. Rollin Hotchkiss first discovered modified cytosine Hotchkiss in 1948 and assumed that this fraction was 5mC because it usually occurs in DNA as
modified cytosine. Although several researchers suggested that DNA methylation/de-methylation could regulate gene expression, several studies showed that DNA methylation was involved in gene regulation and cell differentiation until the 1980s [57, 58]. It was recognized that DNA methyltransferases (DNMTs) are important for mammalian development [59, 60]. DNA methylation, the adding a methyl group to the DNA nucleotide cytosine, is the most studied epigenetic mark [61]. A strictly regulated pattern of DNA methylation is essential for normal in cellular differentiation in higher organisms and plays a vital role throughout life in the regulation and transcription of tissue-specific genes [62]. Research into DNA methylation erasure gained momentum a few years ago when 5-hmC an oxidation product of 5-mC was discovered and further oxidation steps would modify 5-hmC first to 5-fC and 5-caC.

V. ENZYMES INVOLVED

Enzymes involved for catalyzing the process of DNA methylation/de-methylation are following:

1) *DNA Methyltransferases:* DNA methyltransferases enzymes also known as DNMTs are DNMT1, DNMT3A, DNMT3B, and DNMT3L. There are mainly two groups of DMNTs - DNMT1, which replicas the DNA methylation pattern between cell generations during replication (maintenance methylation), and DNMT3a and DNMT3b, which are control in denovo methylation of DNA [64].
2) *Ten-Eleven Translocation (TET):* The three enzymes of the ten-eleven translocation (TET) family are TET1, TET2, and TET3 dioxygenases can gradually oxidize 5hmC to 5fC and 5caC [65, 66]. TET proteins role is modulating the methylation pattern [67], in which TET1 contributed to widespread 5hmC reduction, and TET2 and TET3 might be involved in the mainly formation of downstream cytosine that is de-methylation cascade reaction.
3) *Thymidine DNA Glycosylase*: Thymidine DNA glycosylase (TDG), also an enzyme which is able to remove 5fC and 5caC by activating base-excision repair (BER) and reintroducing of unmethylated cytosine [68, 69]
4) *AID (activation-induced Deaminase):* AID (activation-induced deaminase)/APOBEC (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide) cytidine deaminase family followed by base excision repair [70]. AID exhibits its strongest activity against unmodified cytosine. The deamination of 5-hmC into 5-hmU was suggested through AID & APOBEC, followed by TDG and BER mechanisms [70].
5) Methyl-CpG binding protein 2 (MeCP2): Methyl-CpG binding protein 2 (MeCP2), or methyl-CpG-binding domain (MBD) family– MBD1, MBD2, and MBD4 preferably bind methylated DNA as opposed to MBD3, MBD5, and MBD6 preferring to bind to non-methylated DNA [71], although MeCP2 or MBD proteins are recruited to methylated DNA and prevent the binding of transcription factors.

VI. DNA METHYLATION EFFECT ON *BDNF* EXPRESSION

DNA methylation is a basic epigenetic mechanism for silencing gene through lifecycles [72], and neuronal activity in *BDNF* gene region persuades dynamic changes in DNA methylation. Elevated level of DNA methylation in the promoter area usually promotes a transcriptional repression state [73, 74]. The *BDNF* gene is regulated temporally and spatially by neural activity. Past studies show that the expression of *BDNF* is negatively regulated by DNA methylation and it has also been noted that environmental stimuli can alter DNA methylation levels and therefore levels of gene expression, generating standard DNA methylation level of long-lasting cellular memories required for normal cell functioning.

Previous studies suggest that a mechanism for *BDNF* down-regulation under bad environmental conditions can elevate *BDNF* exon IV DNA methylation. It may be suggest that methylation level increases in diabetic retina known through DNA de-methylation, De novo DNA methylation together with de-methylation of DNA are predominantly considered to be involved in neuronal plasticity [75]. TETs mainly involve in DNA de-methylation pathway and it is the key enzyme of de-methylation (formation of fC & caC).

In this review we mainly target the 5hmc region at the BDNF gene. 5-Hydroxymethylcytosine which is recommended as a DNA nitrogen base of pyrimidine is formed from cytosine which is DNA base by adding a methyl group followed by hydroxygroup; it is considered as important process in epigenetics as group on cytosine i.e. hydroxymethyl group can be able to switch on and off genes. And the function of 5hmc is to regulate gene expression. It was found to be abundant in human and mouse brains. In mammals, the generation of 5hmc occurred by the oxidation of 5-methylcytosine this reaction process is mediated by group of enzymes of TET family. Each mammalian cell seems to be surrounded by 5-Hydroxymethylcytosine, but depending on the type of cells the levels may vary in which the maximum levels are found to be in neuronal cells of the central nervous system [92, 93, 94, 95]

Carey and Xu and their coworkers demonstrate that high levels of glucose can inhibit the conversion of DNA methylation (5mC) to hydroxymethylation (5hmC) by the ten-eleven translocation (TET) dioxygenase family of proteins by influencing α-ketoglutarate levels. Based on their hypothesis, high blood glucose levels would increase 5hmC due to increased TET activity, resulting in gene expression dysregulation [76, 77].

Thus, it was hypothesized in this that over expression of TET in diseased condition may lead to 5hmC level dysregulation in its target genes such as BDNF and this gene contributes to T2DR pathogenesis.

The conversion of 5mC and its oxidized derivatives back to the unmodified state was suggested to occur ‘by de-methylation of DNA which can be either “passive” or “active” de-methylation. “Passive” DNA de-methylation occurs during DNA replication, refers to the failure to maintain patterns of DNA methylation across cell divisions and is thought to result in 5mC replication-dependent of dilution. “Active” DNA de-methylation is independent of replication of DNA and requires enzymes action. TET family proteins have been reported to play critical roles in DNA de-methylation, resulting in 5-mC to 5-hmC alterations [70, 78]. 5-hmC which is a key active intermediate and is further processed by several pathways back to unmodified cytosine direct de-methylation of 5-methylcytosine to cytosine does not occur. All known in vivo conversions of 5mC to cytosine while methylated nucleotide replacement is involved.

DNA de-methylation occurs through two pathways, one is replication dependent de-methylation also known as ―passive de-methylation, and the other is TET enzyme induced ―active de-methylation. DNA base modification is the first step-in this process either TET-mediated oxidation of the methylated base or methylated deamination of AID & TET proteins by the function of dioxygenases activity, iteratively oxidize 5hmC from 5mC and can be further converted to 5fC and 5caC [80], while AID deaminase cytosine to uracil and to a lesser extent 5mC to thymine. Thus, AID could either act directly on 5mC or indirectly by modifying adjacent, ‘regular’ cytosine [79]. Replacement of nucleotides, second active step of DNA de-methylation involves replacing modified nucleotide (possibly together with surrounding nucleotides) via DNA damage repair pathways, primarily through base excision repair. Various glycosylase, such as thymine DNA glycosylase are also involved in this process [79].

TET-mediated 5fC and 5caC are now considered a novel epigenetic DNA modification to promote active DNA de-methylation, representing an active de-methylation pathway [81]. 5-hmC role is generally associated with transcribed genes in regulation of DNA de-methylation and transcription; it is positively correlated with transcription levels and detected in all types of cells. TET proteins are critical DNA de-methylation regulators. Active DNA de-methylation by oxidation is thus initiated by TET and TDG. A subfamily of DNA glycosylases is considered to promote active DNA de-methylation by removing the 5-methylcytosine (unmodified C) base, followed by cleavage of the DNA backbone at the abasic site, and replacing the methylated cytosine with an unmethylated cytosine.

DNA methylation is reversible and dynamic process. DNA de-methylation may be induced by TET convert 5-mC to 5-hmC, 5-hydroxymethylcytosine, that can be further deaminated by AID/APOBEC, produce a DNA mismatch that is repaired by the base excision repair machinery. Recent studies have shown that TET is involved specifically at the neuronal activity-dependent *BDNF* in inducing DNA de-methylation [78]. DNA methylation is heritable which can pass from cell to cell. Methylated genes can use as biomarkers for early retinopathy detection because methylation changes often occur early in the disease. Hypermethylated gene detection could identify tissues from increased risk patients. In addition methylation's reversible nature offers the potential to reverse aspects of the appropriate therapy [82].

VII. TEN-ELEVEN TRANSLOCATION PROTEIN:

TET enzyme family -TET1, TET2, and TET3 have a place with the Fe2+- and 2-oxoglutarate (2OG)-dependent dioxygenases superfamily. Each of the three TET proteins has a highly conserved carboxy-terminal catalytic region consisting of a cysteine-rich domain (Cys-rich) and a double-stranded β-helix (DSBH) domain [78, 83]. TET proteins are known to have significant regulatory roles. Starting with research on DNA methylation patterns TET proteins and their role in regulating DNA methylation/de-methylation patterns, TET proteins alter the DNA methylation status by catalyzing the 5mc methyl group's consecutive oxidation to form 5hmC, which in turn is further oxidation by TET proteins into 5fC and 5caC [67, 78, 84, 85].

TET enzyme initiated DNA de-methylation pathways i.e. active de-methylation and passive de-methylation. DNA methylation (5mC) is catalyzed by DNMTs, which can be oxidized to 5hmC, 5fC, and 5caC by TET proteins. All adjustments can be lost by passive de-methylation, a replicative loss due to lack of maintenance during cell division. Active de-methylation can occur as thymine-DNA glycosylase can excise 5fC and 5caC, which can be further repaired through the base excision repair pathway. TET1, Dnmt1, and Dnmt3a are the primary enzymes associated with DNA de-methylation pathway, 5fC and 5caC can be further excised by thymine-DNA glycosylase (TDG). TET1 (T) can replace multiple transcription factors during cell reprogramming [85]. The description of inactivating mutations in TET2 recommends that the deregulation of this 5-mC conversion causes cellular transformation in part.

These proteins affects several aspects of human life—including cell growth regulation, embryonic stem cell maintenance, and cell differentiation—as well as a number of mutations leading to a large number of diseases, such as those caused by chromosomal translocations and those leading to DR with a focus on TET enzymes and methylation regions mC, hmC, fC, caC, and hmU binding Efficiency. During cytosine modification TET2 can change over 95% of the 5mC to 5hmC (~60%), 5fC (~30%), and 5caC (5%), but it can only convert about 40% or 25% when 5hmC or 5fC containing DNA was utilized as a substrate [85].

Thus, these mutations affect TET leading to partial or all-out gene inactivation. TET enzymes are also inhibited by metabolic disturbances resulting from gene mutations that encode isocitrate dehydrogenase (IDH), fumarate hydratase (FH) or succinate dehydrogenase (SDH) including DNA de-methylation. Deregulation of DNA methylation may also be achieved directly through mutations in genes encoding DNMT [86, 87]. These enzymes are also involved in the citric acid cycle, are frequently mutated in epigenetic alteration de-methylation protein TET, and lead to the production of alpha-ketoglutarate, which is used as a cofactor for TET and is necessary for its activity.

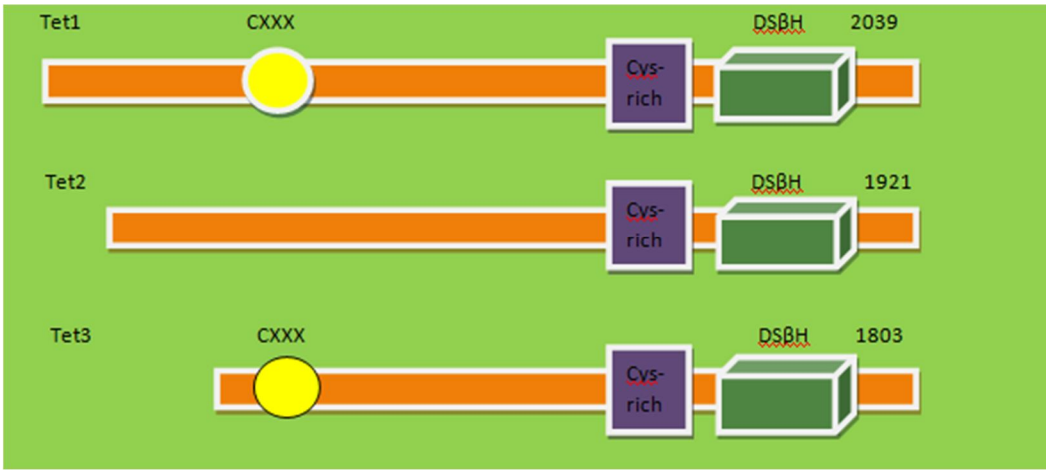

Figure 3. Structure of TET protein [78]

TETs family of protein consists of a catalytic domain (CD) that harbors a DSBH domain and a Cys-rich domain. At the amino terminal of TET1 and TET3.Three conserved domains including CXXC zinc finger, TET2 does not contain a predicted CXXC domain (fig.) 3. CD, Catalytic domain; double-stranded b-helix (DSBH) fold of the 2OG-Fe (II) dioxygenases domain are indicated. Cysteine-rich region (Cys-rich), numbers represent the amino acid numbers [78]. In addition to the catalytic domains, the CXXC domains also participate in the regulation of TET-mediated gene expression. TET family proteins share structural characteristics of highly homologous protein. TET mainly involved in the formation of downstream cytosine intermediates (5fC and 5CaC), 5hmC mainly accumulates in introns, and these results might indicate that 5hmC removal from introns is partly due to the de-methylation function of TET [88].

Abnormal glucose levels increase cellular oxidation, which triggers the progression of TET enzyme-assisted DNA demethylation, resulting in epigenetic dysregulation of damaged tissues. (89) Studies show that, the diagnosis of type 2 diabetic retinopathy with the help of regulating the level of 5hydroxymethylcytosine. TET enzyme methylation activity can be inhibited by the use of appropriate methylation inhibitors, which in turn can decrease hypermethylation of bdnf protein. (90)

VIII. CONCLUSION

Over the past few years, the role of TET protein and 5hmC has been widely studied in many disease developments. Regular DNA methylation pathway may manage the level of BDNF protein as TET dysregulation is related to BDNF gene dysregulation, which is important factor to prediction of Type 2 Diabetic Retinopathy. Reduced BDNF level can cause retinal cell death in a diseased condition like Type 2 Diabetic retinopathy. In Type 2 Diabetic retinopathy increased glucose levels cause cellular oxidation, which triggers the process of DNA demethylation process supported by TET family of protein, resulting in epigenetic dysregulation of the damaged tissues. By using suitable regulator we can regulate the higher activity of TET family of proteins that can further regulate the level of 5hmc, which in turn may help in properly regulate the expression of *BDNF* gene. By the regulating the BDNF gene, retinal neurons can be protected from hyperglycemia via the TrkB/ERK/MAPK pathway and provides novel insights into of Type 2 Diabetic Retinopathy pathogenesis.

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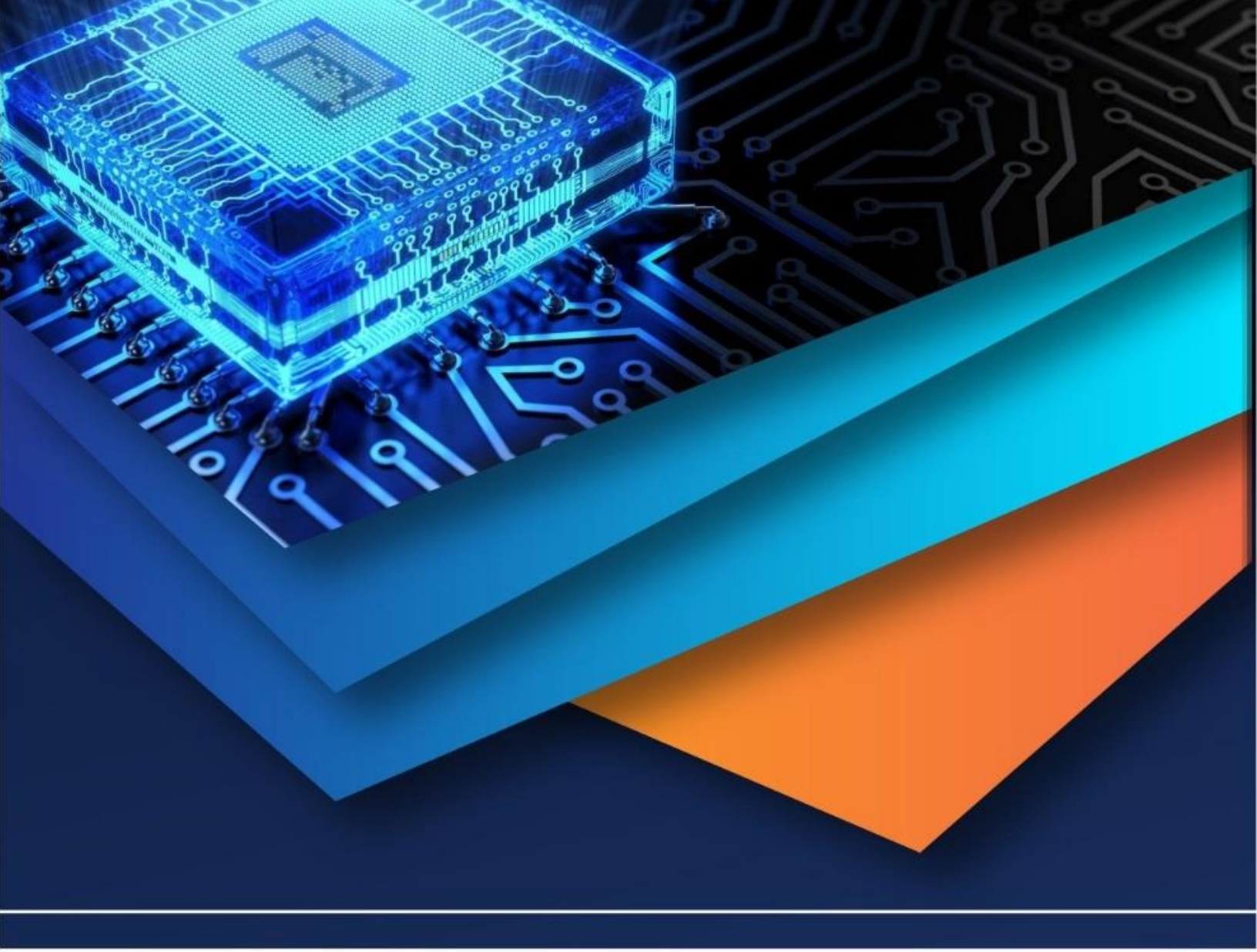

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