Epigenetic Modification of Ten-Eleven Translocation Protein Mediated DNA Methylation/De-Methylation in 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 most common complications of diabetes that affect the blood vessels of the retina, leading to blindness. Type 2 Diabetic Retinopathy is a term used for all the abnormalities of the small blood vessels of the retina caused by diabetes. Interaction between genetic and environmental factors in disease development most likely involves epigenetic modifications. The study so far suggested that epigenetics mechanism also play a key role in the pathogenesis of Type 2 Diabetic Retinopathy. BDNF is a neurotrophic factor, previous studies reported that reduced level of BDNF in the diabetic retina may damage neurons, thereby leading to neurodegeneration. The number of studies on various factors such as Vascular Endothelial Growth Factor (VEGF) has been done to prevent Type 2 Diabetic Retinopathy by different mechanisms. Till date, there are no comparable studies available investigating the significance of TET mutations in DNA methylation/de-methylation in BDNF gene, to our knowledge, this is the first report of BDNF methylation/de-methylation status with respect to T2DR. The main concept of the present review focus on epigenetic modification of Ten-Eleven Translocation protein mediated DNA methylation/de-methylation in Brain Derived Neurotrophic Factor for the Pathogenesis of Type 2 Diabetic Retinopathy. BDNF protects retinal neuron from hyperglycemia through the TrkB/ERK/MAPK pathway was reported in past studies. Aim of this review is to study the effect of TET mediated epigenetic modification such as DNA methylation/de-methylation on BDNF gene in case of Type 2 Diabetic Retinopathy.**

***Index Terms*- Brain derived neurotrophic factor, Epigenetics, Ten- Eleven Translocation protein, Oxidative stress, Retinopathy**

I. INTRODUCTION

Type 2 Diabetic Retinopathy (T2DR) is one of the most common complications of diabetes, and is the important cause of blindness in developed countries. After 15 years with diabetes, approximately 80% of patients have retinopathy particularly, the prevalence of type 2 diabetes (T2D) is increasing at an alarming rate [1, 2]. It is affected in young adults over 90% patients with 20 years of diabetes. The disease carries a heavy problem on our society as it is responsible for 4.8% of the 37million cases of eye disease related blindness worldwide, the number of people with diabetic retinopathy is expected to grow from 126.6 million in 2010 to 191.0million by 2030 [3]. T2DR is one of the most common complications of diabetes that affect the blood vessels of the retina, leading to blindness. T2DR is a term used for all the abnormalities of the small blood vessels of the retina caused by diabetes. Interaction between genetic and environmental factors in disease development most likely involves epigenetic modifications [4], role of epigenetic mechanisms in the etiology of these disorders and related metabolic abnormalities such as obesity, dyslipidemia, hyperinsulinemia, are often associated with T2D. Type 2 diabetes is characterized by insulin resistance in insulin-targeting tissues, mainly the skeletal muscle, liver, and adipocytes. It is a complex disorder to chronically elevated blood glucose level. Recent study suggests epigenetics

mechanism also plays a key role in the pathogenesis of DR. That epigenetic factors, including DNA methylation and histone modification, may affect the susceptibility for T2DR [4]. In the mammalian genome, DNA methylation is relatively fixed and is regarded as a bonafide epigenetic modification [5, 6], involving the transfer of a methyl group onto the C5 position of the cytosine to form 5-methylcytosine (5mC), several molecular mechanisms, including methylation of cytosine within CpG dinucleotide [7], molecular mechanisms linking environmental factors and T2D still remains limited. This review focus on epigenetic mechanisms, DNA methylation/de-methylation and discuss about the established and emerging roles of Ten Eleven Translocation (TET) protein in bdnf gene associated with T2DR.The prevalence complications of diabetes are strongly related to the duration of retinopathy, Diabetes is a disease of abnormal glucose metabolism resulting in hyperglycemia (high blood glucose) due to either a deficiency of insulin secretion or insulin resistance due to pathologic changes that involve small and large blood vessels, nerves, skin, and the lens of the eye. Diabetes and metabolic disorders are leading causes of micro and macro vascular complications, micro vascular complications of diabetes include retinopathy, nephropathy, neuropathy, and thought the result of an abnormal thickening of the basement membrane of the capillaries [8].

Most importantly, diabetes is a polygenic multifactorial disease and group of diseases, studies was suggest epigenetic factors (DNA methylation/de-methylation and histone modification) play a major role in the pathogenesis of this multifactorial disease and its complications, may affect the susceptibility for type 2 diabetes (T2D) and the progression of theirs complications [9]. Epigenetic alterations play a role in pathologic responses such as neurodegeneration and inflammation which contribute to the progression of diabetic retinopathy [10]. Thus, diabetes has become a major public health problem. T2DR is a leading cause of blindness in the western world. T2DR has characteristics of chronic inflammatory disease, microvascular and neurodegenerative disease, and the blood-retinal barrier breakdown is a hallmark of this disease. The structures and functions of the eyes are complex, all structures in the eye, the neurosensory retina has been the most widely studied In short, the retina can be divided into two parts neural retina (NR) and retinal pigment epithelium (RPE) [11]. T2DR has been considered a micro vascular disease, but multiple cell types in the retina are affected by T2DR for example, neurons and glial cells [12]. OS has been usually regarded as the key factor for the emergence of ocular disease and has been involved in increased vascular permeability, disruption of blood-retinal barrier, micro vascular abnormalities, apoptotic loss of retinal capillary cells, involves of micro aneurysms, hemorrhages and retinal edema as well as neovascularization in some cases, retinopathy may result in the vision loss. T2DR is a highly specific vascular complication of both type-I and type-II diabetes [13]. Retinopathy is divided into two major categories; Proliferative diabetic retinopathy (PDR), characterized by neovascularization (growth of new blood vessels on the retina) and Non-proliferative diabetic retinopathy (NPDR), characterized by increased vascular permeability, it is advance stage of retinopathy. In vivo and in vitro studies were identified five major biochemical pathways proposed as potential links between how hyperglycemia produces DR:

1. The polyol pathway,
2.Advanced glycation end products (AGEs) pathway,
3. The hexosamine pathway,
4. The protein kinase C pathway, as well as
5. Poly (ADP-ribose) polymerase activation [14, 15].

Together these pathways produce oxidative stress (OS), inflammation, micro vascular dysfunction and mitochondrial damage, which in turn up-regulate pro-inflammatory mediators, transcription factors, chemokine's, and adhesion molecules [16]. Hyperglycemia stimulates protein kinase C (PKC), polyol pathway, formation of advanced glycation end products (AGEs) and cellular oxidative stress [17]. OS plays a pivotal role in the development of diabetes complications, both microvascular and cardiovascular. These factors then compromise the blood-retinal barrier (BBR) and lead to the up-regulation of pro-angiogenic growth factors and hormones that produce DME and PDR [18], recent findings highlight the important contribution of dysfunction and death of retinal neurons in DR to changes to visual function including the loss of contrast sensitivity, changes in color perception, visual field defects, and abnormal dark adaptation [19-21].

Studies have suggested that brain-derived neurotrophic factor plays a role in glucose and lipid metabolism and inflammation. BNDF expression is also regulated by epigenetic chromatin remodeling, including DNA methylation of cytosine's in cytosine-guanine (CpG) dinucleotide. An increase in CpG methylation at promoter regions on the BDNF gene have been found to be correlated with decreased neuronal synthesis of BDNF. Till date, there are no comparable studies available investigating the significance of TET mutations in DNA methylation/de-methylation in BDNF gene, To our knowledge, this is the first report of BDNF methylation/de-methylation status with respect to T2DR.The main concept of the present review provide new mechanistic insights into the role of TET mediated epigenetic modification change of DNA methylation/de-methylation in BDNF gene, most likely diseases development, and drug designing, regenerative therapies. The aim of this study was to evaluate the relationship between serum BDNF levels and various metabolic parameters and inflammatory markers in patients with T2DR. Thus, understanding and characterizing the epigenetic regulators and their role in the pathogenesis of type2 diabetic retinopathy might help identify novel targets to conflict this disease which is the major cause of blindness in young adults.

II EPIGENETICS

The term epigenetic was introduced by Conard Waddington in 1942 as a concept of environmental affect in inducing phenotype modification [22]. Epigenetics is the study of mitotically heritable alterations in gene expression potential that are not mediated by changes in DNA sequence. Genetics is the study of heritable changes in gene activity or function due to the direct alteration of the DNA sequence. Such alterations include point mutations, deletions, insertions, and translocation. In contrast, epigenetics is the study of heritable changes in gene activity or function that is not associated with any change of the DNA sequence itself [23]. Epigenetic modifications, as well as methylation, acetylation, phosphorylation, and ubiquitina¬tion, alter the interaction between the DNA, histones and nuclear proteins, thus affecting gene transcription and regulate gene silencing or expression [24]. Epigenetic mechanisms have established them as key players in several cellular processes including cell differentia¬tion, DNA replication repair and aging [25-28]. This epigenetic mechanisms play a major role in the developmental origins of health and disease [29]. Epigenetic regulation is critical for mammalian development and cellular differentiation, and small changes in the epigenome (epigenetic dysregulation) causes wide range of adult-onset chronic diseases [30]. Although there is uniform definition of epigenetics, described as "heritable variations in gene function that occur without a change in the nucleotide sequence" [31]. Epigenetic mechanisms include DNA methylation/de-methylation, noncoding RNA activity and histone modifications to regulate gene expression [32-35]. 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 Modifications in Diabetes retinopathy; It has become clear that diabetic background interrupts metabolic homeostasis and also alters various genes, including genes associated with oxidative stress, inflammation and apoptosis [36-39], both genetic susceptibility and environmental factors play critical roles in the development of metabolic diseases including obesity and DR. Epigenetics mentions to gene expression alterations that are unrelated to DNA sequence changes, which can be inherited and affected by environmental factors [40]. To control gene expression being modified by transcriptional and translational initiation, can also be controlled by changes of the genes without altering the nucleotide composition of the genome [31]. Recent studies have shown that epigenetic changes play a major role in many chronic diseases such as diabetes and its complication.

III. BRAIN DERIVED NEUROTROPHIC FACTOR

BDNF is a member of the neurotrophin family, which consist of nerve growth factor, neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) [41]. The role of BDNF in cell differentiation (maturation), synaptic connectivity, neural growth, and maintenance of target neurons is well established; it has also been involve, in synaptic plasticity of brain function, such

as learning and memory [42,43]. BDNF has been shown to regulate the growth of plasticity and survival of dopaminergic, serotonergic and cholinergic neurons also, it is involved in the pathogenesis of a wide range of psychiatric disorders, such as bipolar disorders, major depression, and regulates glutamatergic neurotransmitter release and promotes the development of GABAergic neurons. BDNF is widely expressed through the mammalian brain [44]. This makes BDNF a key factor in learning and memory, cognitive function, reward-related processes and circuit formation. It was also implicated BDNF in anxiety-like behaviors and have shown a decreased BDNF expression in response to different types old diseases [45].

Neurotrophin represent a small family of structurally and functionally related growth factors that in mammals also include BDNF, its acting via the protein tyrosine kinase receptor (TrkB) is involved in a number of functional processes of the brain, including memory formation, and neuronal connectivity, it promotes the development of immature neurons and enhances the survival of adult neurons [46]. The bdnf gene is located in a region of chromosome 11 which is the short (p) arm of chromosome 11 at position 14.1 (cytogenetic Location: 11p14.1) the human BDNF has a complex gene structure, inclosing 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. All BDNF mRNAs contain the sequence for the pro-BDNF protein, encoded by exon IX. Molecular Location of BDNF gene: base pairs 27,654,893 to 27,722,058 on chromosome 11 (homo Sapiens) [47].

IV BDNF & T2DR

Brain-derived neurotrophic factor (BDNF) is associated with systemic inflammatory conditions, such as diabetes [48]. Human studies suggested that BDNF may contribute to glucose metabolism and have a pathogenic role in the development of type 2 diabetes mellitus (T2DM) in human serum BDNF levels were significantly lower in patients with advanced T2DM as compared to normal patients [49]. For diagnosis of T2DM, serum BDNF levels as an indicator, based on the receiver operating characteristic (ROC) curve. BDNF can be seen as an indicator of independent diabetes complications study suggested that low levels of BDNF accompany impaired glucose metabolism. Importantly, study found that decreased BDNF were correlated with obesity and diabetes complications [50]. Numerous studies reported serum levels of BDNF in diabetic patients and in diabetic animals, which correlated with insulin resistance, reduced glucose and lipid metabolism, serum BDNF levels were inversely correlated with fasting glucose. Another words reduced levels of BDNF in the serum of diabetic retinopathy patients and in the retina of diabetic rats [50].

BDNF is a member of the neurotrophin family of growth factors and is important for the differentiation and development, studies was demonstrated that at initial stage of DR, specific retinal ganglion cells (RGCs) undergo apoptosis and retinal neurodegeneration is likely to be associated with a lack of BDNF [51]. BDNF is critical for photoreceptor cells and the repair of damage to the retina and the optic nerve. BDNF is expressed in RGCs and muller glia in the retina [52]. BDNF promotes survival in injured RGCs [53] and also promotes regeneration of the nerve fiber [54,55]. In addition, BDNF promotes the survival of retinal interneu¬rons and is important for establishing phenotypes and synaptic connections in the developing retina [56]. BDNF has been reported to inhibit neuroretinal cell death under conditions of cardiac ischemia, and to inhibit apoptosis in rat RGCs at initial stages of DR [57]. However, the mechanisms by which BDNF regulates RGCs remain unclear [58-60]. TrkB is a receptor BDNF protein involved in the development and maturation of the central and peripheral nervous systems. Studies have demonstrated in vivo that the death of retinal neurons at early stages of T2DR correlates with decreased levels of BDNF, and TrkB receptor is essential for the defense of brain neurons and RGCs induced by BDNF. However, it is unknown whether BDNF protects retinal neurons exposed to hyperglycemia in vitro or whether the ERK/MAPK pathway is activated in response to BDNF induced neuro protection. The cells exposed to hyperglycemia exhibited higher levels of apoptosis, while BDNF inhibit apoptosis mediated by hyperglycemia the

protective effects of BDNF on cells was a positive correlation between the BDNF and the survival of neuron in diabetic eye.

V DNA METHYLATION

It is a major epigenetic mechanism involving direct chemical modification to the DNA called DNA methylation. Methylation of DNA can change the functional state of regulatory regions, but it does not alter the Watson–Crick base pairing of cytosine. It thus presents the standard 'epigenetic' mark and is functionally involved in many forms of stable epigenetic repression, such as imprinting, and silencing of repetitive DNA [61]. DNA methylation is a "transfer of a methyl group onto the C5 position of the cytosine to form 5-methylcytosine (5mC)", transfer of methyl group with the help of enzyme S-adenyl methionine (SAM) to form 5mC.

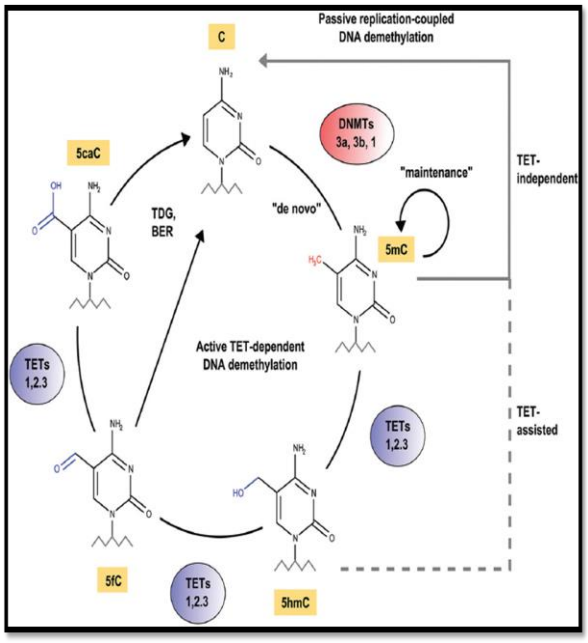

Figure 1. DNA methylation pathway [70]

DNA methylation and de-methylation pathway, DNMTs catalyze the methylation of cytosine by transfer of methyl group to the position C5. TET enzymes can catalyze the oxidation of 5-methylcytosine to 5-hydroxymethylcytosine, further help of TET enzymes, in 2009, two more cytosine analogs have been discovered, TET-dependent oxidative reactions lead to 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. Dilution of modified cytosines, 5mC or 5 hmC, during DNA replication 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. [70]

It is considered as one of the major epigenetic modification; methylation of the CpG islands, a CG rich region in the promoter of many genes, changes protein-DNA interactions leading to alterations in chromatin structure, and this affects the binding of transcriptional mechanism, resulting in gene suppression [62, 63]. In 1948, Rollin Hotchkiss first discovered modified cytosine Hotchkiss hypothesized that this fraction was 5mC because it modified cytosine occur usually in DNA. Although many researchers proposed that DNA methylation/de-methylation might regulate gene expression, it was not clarify until the 1980s that several studies demonstrated that DNA methylation was involved in gene regulation and cell differentiation [64, 65]. DNA methyltransferases (DNMTs) were recognized as being essential for mammalian development [66, 67]. The most studied epigenetic mark is DNA methylation, the attachment of a methyl group to the DNA nucleotide cytosine [68]. A strictly regulated DNA methylation pattern is essential for normal in cellular differentiation in higher organisms, and has a key role throughout life in tissue specific gene regulation and transcription [69]. Research into DNA methylation erasure gained momentum a few years ago with the discovery of 5-hmC an oxidation product of 5-mC and further oxidation steps would modify 5-hmC first to 5-fC and 5-caC. The role of this new epigenetic modification in TET dependent DNA de-methylation and other potential epigenetic roles.

Enzymes involve in DNA methylation/de-methylation pathway; The process of DNA methylation/de-methylation catalyzed by the family of enzymes these are;

- DNA methyltransferases enzymes which are known as DNMTs; DNMT1, DNMT3A, DNMT3B, and DNMT3L [71]. There are 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 novo methylation of DNA [72].

- The three enzymes of the TET family; TET1, TET2 and TET3 dioxygenases can further oxidize 5hmC to 5fC, and 5caC in a stepwise manner [73,74] roles of TET proteins in modulating the methylation pattern [75], TET1 contributed to widespread 5hmC reduction, TET2 and TET3 may be mainly involved in the formation of downstream cytosine that is de-methylation cascade reaction.
- Thymidine DNA glycosylase (TDG), is then able to remove 5fC and 5caC, triggering base-excision repair (BER) activity and the reintroduction of unmethylated cytosine [73,76]
- AID (activation-induced deaminase)/APOBEC (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide) family of cytidine deaminases followed by base excision repair [77]. AID exhibits its strongest activity against unmodified cytosine. It has been suggested that the deamination of 5-hmC into 5-hmU occurs via AID & APOBEC, followed by TDG and BER mechanisms [77].
- Methyl-CpG binding protein 2 (MeCP2), or methyl binding domain (MBD) family– MBD1, MBD2 and MBD4 preferentially bind methylated DNA in contrast to MBD3, MBD5 and MBD6 that prefer to bind to non-methylated DNA [78], although MeCP2, or MBD proteins are recruited to the methylated DNA and prevent the binding of transcription factors.

Impact of DNA methylation on BDNF expression; DNA methylation is a fundamental epigenetic mechanism for gene silencing throughout lifecycles [79], Neuronal activity induces dynamic DNA methylation changes in the BDNF gene region. Increased DNA methylation at the promoter area normally promotes a state of transcriptional repression [80, 81]. The BDNF gene is regulated by neural activity in a temporal and spatial manner study prove that DNA methylation negatively regulate the expression of BDNF. It has been reported that environmental stimuli may alter the levels of DNA methylation and consequently gene expression, generating long-lasting cellular memories standard level of DNA methylation required for normal cell functioning.

Emerging evidence suggests that elevated BDNF exon IV DNA methylation is a mechanism for BDNF down-regulation in adverse environmental conditions. It may be suggest that methylation level increases in diabetic retina known through DNA de-methylation, De novo DNA methylation together with DNA de-methylation are predominantly thought to be implicated in neuronal plasticity [82]. TETs involve in DNA de-methylation pathway, TET is a main enzyme of de-methylation (formation of fC & caC) Thus, in this study, it was hypothesized that overexpression of TET may lead to down regulate, its target genes such as BDNF which will contribute to the pathogenesis of T2DR.

VI DNA DE-METHYLATION

The conversion of 5mC and its oxidized derivatives back to the unmodified state has been proposed to occur by DNA de-methylation can occur either “passive” or “active” de-methylation. “Passive” DNA de-methylation takes place during DNA replication when the newly synthesized strand is not methylated it refers to the failure to maintain DNA methylation patterns across cell divisions and is believed to result in replication-dependent dilution of 5mC. “Active” DNA de-methylation is independent of DNA replication and requires the act of enzymes, it was reported that TET family proteins play critical roles in DNA de-methylation by converting 5-mC to 5-hmC [77, 83]. A key active intermediate, 5-hmC is further processed by several pathways back to unmodified cytosine direct de-methylation of 5-methylcytosine to cytosine does not occur. Instead, all known in vivo conversions of 5mC to cytosine involve replacement of the methylated nucleotide.

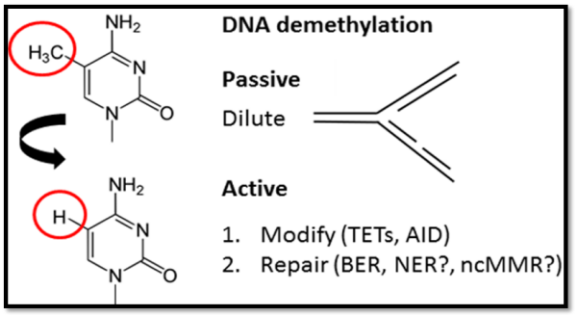

Figure2. Active DNA de-methylation: A two-step process [84]

There are two pathways involved in DNA de-methylation: one is replication‐dependent de-methylation, known as "passive de-methylation," and the other is TET enzyme‐induced "active de-methylation." The first step in this process is DNA base modification: either TET-mediated oxidation of the methylated base or deamination of the methylated by AID & TET proteins through the function of dioxygenase activity, TETs could iteratively oxidize 5hmC is generated out of 5mC and can be further converted to 5fC and 5caC, [85], AID on the other hand, deaminates cytosine to uracil and – to a lesser extent – 5mC to thymine. In that way, AID could either act directly on 5mC or indirectly by modifying neighboring, 'regular' cytosine's [84]. The second step of active DNA de-methylation is nucleotide replacement: The modified nucleotide (possibly together with surrounding nucleotides) is replaced via DNA damage repair pathways, presumably mainly by base excision repair. Various glycosylases, such as thymine DNA glycosylase are involved in this process. Despite recent progress in deciphering these active DNA de-methylation pathway [84].

Another words TET-mediated 5fC and 5caC formation and promote active DNA de-methylation, representing an active de-methylation pathway, is now considered a novel epigenetic DNA modification [86]. 5-hmC role in regulating DNA de-methylation and transcription is generally associated with transcribed genes, it is positively correlated with transcription levels and detected in all cell types, TET proteins are critical regulators for DNA de-methylation. TET and TDG thus initiate active DNA de-methylation by oxidation. A subfamily of DNA glycosylases are 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 the methylated cytosine is replaced by an unmethylated cytosine.

DNA methylation is reversible; DNA methylation is a dynamic and reversible 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. TET has been recently shown to induce DNA de-methylation specifically at the neuronal activity-dependent BDNF [83]. DNA methylation is reversible because it does not alter the DNA sequence; however, it is heritable from cell to cell. Methylated genes can serve as biomarkers for early detection of retinopathy, because methylation changes often appear early in disease, detection of hypermethylated genes could identify tissues derived from patients with increased risk. Furthermore, the reversible nature of methylation offers the potential to revert aspects of the appropriate therapy [87].

VII TEN-ELEVEN TRANSLOCATION PROTEIN

Enzymes of TET family (TET1, TET2 and TET3) belong to the superfamily of Fe2+- and 2-oxoglutarate (2OG)-dependent dioxygenases all three TET proteins possess a highly conserved carboxy-terminal catalytic region that is composed of a cysteine-rich (Cys-rich) and a double-stranded β-helix (DSBH) domain [83,88]. TET proteins are known to have important regulatory roles. Initial studies of DNA methylation patterns TET proteins and their role in regulation of DNA methylation/de-methylation patterns, TET proteins modify the methylation status of DNA by catalyzing consecutive oxidation of the methyl group of 5mC to form 5hmC,which in turn undergoes further oxidation by TET proteins into 5fC and 5caC [74,83,89,90].

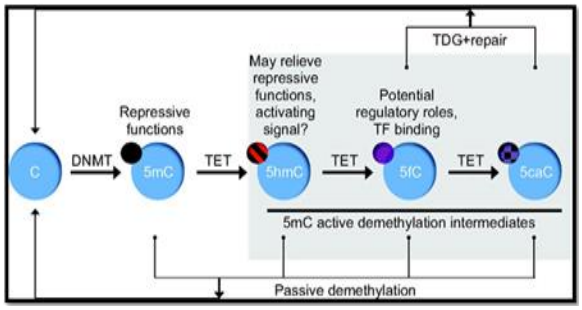

Figure 3, Iterative oxidation of 5methylcytosine TET dependent manner and de-methylation pathways [91]

TET enzyme‐induced DNA de-methylation pathways i.e. active de-methylation and passive de-methylation. DNA methylation (5mC) is generated by DNMTs, which can be oxidized by TET proteins to generate 5hmC, 5fC and 5caC. All modifications can be lost through passive de-methylation, which is replicative loss due to lack of maintenance during cell division. Active de-methylation can also 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 main enzymes involve in 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 [90]. The description of inactivating mutations in TET2 suggests that cellular transformation is in part caused by the deregulation of this 5-mC conversion.

The impact of these proteins reaches 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 multitude of diseases, such as those induced by chromosomal translocations and those that lead to DR with an emphasis on TET enzymes and methylation regions mC, hmC, fC, caC, and hmU binding Efficiency. During cytosine modification TET2 is able to convert more than 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 used as a substrate [90].

These mutations affect TET and result in partial or total inactivation of the gene. Metabolic perturbations resulting from mutations in genes encoding isocitrate dehydrogenase (IDH), fumarate hydratase (FH) or succinate dehydrogenase (SDH) also inhibit the TET enzymes and, in turn, DNA de-methylation. Deregulation of DNA methylation may also be achieved directly through mutations in genes encoding DNMT [92, 93]. Might also be new avenues for research, these enzymes are 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 required for its activity.

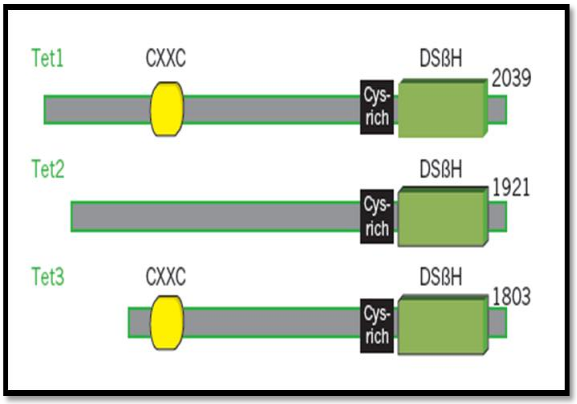

Figure 4. Structure of TET proteins [83]

TETs family of protein include a 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.). CD, Catalytic domain; double-stranded b-helix (DSBH) fold of the 2OG-Fe (II) dioxygenase domain are indicated. Cysteine-rich region (Cys-rich), numbers represent the amino acid numbers [83]. Besides the catalytic domains, the CXXC domains are also involved in TET-mediated gene expression regulation. TET family proteins share highly homologous protein structural features. TET mainly involved in the formation of downstream cytosine intermediates (5fC and 5CaC), 5hmC mainly accumulates in introns, these results might indicate that 5hmC removal from introns is partly due to the de-methylation function of TET [94].

VII. CONCLUSION

In our opinion, TET mediated DNA methylation/de-methylation is one of the most important epigenetic event of methylation, modification pathway that has been associated in the regulation of numerous biological processes such as cell development, and cell differentiation and normal level of BDNF is effective in protecting retinal cells from hyperglycemia which is an approach towards new drugs and gene therapies as well as personalized medicine to prevent or slow down the progression of Type 2 Diabetic Retinopathy.

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