



Epigenetic Modification of Ten Eleven Translocase Enzyme Mediated Hydroxymethylcytosine in Brain Derived Neurotrophic Factor for the Pathogenesis of Type II Diabetes Retinopathy

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Abstract

Epigenetic modulations play essential roles in diverse biological processes. During the past several years, DNA demethylation has been discovered in embryonic and postnatal development. Although some potential functions of DNA methylation have been demonstrated already, many questions remain in terms of unveiling the role of 5hmC; whether it serves either merely as an intermediate of DNA demethylation or as a stable epigenetic marker. 5-hydroxymethylcytosine (5hmC) is proved to be not merely serving as an intermediate of DNA demethylation, but also acts as a stable epigenetic marker. This review defines how to control the gene expression and DNA Methylation in the CpG islands and, The DNA Demethylation leads to convert the 5mC to 5hmC by the TET protein and again the 5hmC transfer to 5fC by the TET enzyme in the BDNF gene. This review shows the method to analyze the DNA Methylation at the 5hmC region for the BDNF gene and the fully mechanism which shows the demethylation from 5hmC to 5fC and the pathway of DNA Demethylation from cytosine to 5CaC (Carboxyl acetyl cytosine)

This review summarizes the current knowledge of the function of 5hmC in brain and the focus on the Diabetes Retinopathy. The role of 5hmC in the BDNF gene in Diabetes Retinopathy plays an important role and it might be helpful for therapeutic purpose.

Keywords-*Epigenetic Modification, DNA Methylation, Brain Derived Neurotrophic Factor, TET Protein.*

Introduction

Epigenetic mechanisms change the gene expression and function without altering the base sequence of DNA and may be reversible, heritable, and influenced by the environment^[6]. Epigenetic modifications include DNA methylation, histone modifications, chromatin remodeling,

and distribution of non coding RNA^[6]. Control of gene expression is defined as the ability of a gene to form a purely active protein in mammals, in addition up to individual modulation through transcriptional and translational beginning, can also be inhibited by change “on the top” of the genes without altering the nucleotide composition

of the genome^[7]. These “epigenetic” modification are constant, but reversible, and be conceded from generation to generation. latest study have indicate that epigenetic changes play a major role in many chronic diseases such as cancer and diabetes where small changes in the epigenome over time are considered to lead to disease manifestation^[8]. Three main epigenetic mechanisms considered to control gene expression are DNA methylation, histone modifications, and noncoding RNA activity These epigenetic modification take part in the DNA methylation and convert the mc to 5hmc^[6]. Epigenetic modification take part in three different process –DNA Methylation, Histone Modification and RNA Interference. The main DNA methylation is procedure in which methyl groups are added to DNA. Methylation which customize or changes the function of the DNA. When posted in a gene promoter, DNA methylation typically acts to control gene transcription^[9]. DNA methylation at the fifth carbon of cytosine (5-methylcytosine,5mC) is the best studied epigenetic modification, and plays pivotalroles in multiple biological processes^[10]. The enzymes that establish and maintain the landscape of DNA methylation, namely the DNA methyltransferases (Dnmts), have three family-embers, Dnmt1 (preserving the methylation), Dnmt3A and 3B (denovomethyltransferase) and a regulatory subunit Dnmt3L DNA methylation also define as addition of a methyl group on fifth position of cytosine residues of the cluster of CpG dinucleotides (CpG Island), This CpG regulatory sites of most genes, is linked with transcriptional repression^[11]. DNA methylation induce and reinforce the development of heterochromatin, which is a strongly packed figure of chromatin related with the suppression of transcription The most widely-studied regulatory methylation occurs at gene promoters, in CpG-richregions termed ‘CpG islands’ where it induces a transcriptionally silent epigenetic state that is inherited faithfully in descendant cells^[13]. These DNA methylation methylate the mc to 5hmc by the tetprotein^[14].

This review shows the changes in the BDNF gene at the 5HMC region in the DNA Methylation. DNA Methylation take part in the BDNF gene and change the mc(methyl cytosine) to 5 hmc (hydroxymethyl cytosine) likewise by the TeT protein the 5hmc transfer into the 5fc (formyl cytosine).BDNF is define as the BDNF-brain-derived neurotrophic factor (BDNF), the second member of the “neurotrophic” family of neurotrophic factor^[19].The BDNF gene has four 5exons that are associated with distinct promoters, and one 3’ exon (exon V) that encodes the mature BDNF protein.BDNF is released by either a nerve cell or a support cell, such as an astrocyte, and then binds to a receptor on a nearby nerve cell This binding results in the production of a signal which can be transported to the nucleus of the receiving nerve cell^[20]. There, it prompts the increased production of proteins associated with nerve cell survival and function^[21].This review mainly focuses on the 5hmc region at the BDNF gene.5hmc-5-Hydroxymethylcytosine is a DNA pyrimidine nitrogen base. It is formed from the DNA base cytosine by adding a methyl group and then a hydroxygroup^[15]. It is important in epigenetics, because the hydroxymethyl group on the cytosine can possibly switch a gene on and off^[13]. it was found to be abundant in human and mouse brains. In mammals, it can be generated by oxidation of 5-methylcytosine, a reaction mediated by the Tet family of enzymes^[16]. Every mammalian cell seems to contain 5-Hydroxymethylcytosine, but the levels vary significantly depending on the cell type^[17]. The highest levels are found in neuronal cells of the central nervous system. The function of the 5hmc is it may regulate gene expression or prompt DNA demethylation^[18]. BDNF is mainly present in the Brain and the retina of eyes so our main focus on the Type II Diabetic Retinopathy.Hyperglycemia is the mainly cause of Diabetes retinopathy and it lead to intracellular sorbitol accumulation, so resulting in osmotic stress, which plays an essential role in the development of diabetic retinopathy^[1]. *Type II*

Diabetes retinopathy is one of the most common complication of diabetes that affect the blood vessels of the retina, leading to blindness. Diabetic retinopathy is a term used for all the abnormalities of the small blood vessels of the retina reason by diabetes, this lead to weakening of blood vessel walls or leakage from blood vessels. *Type II* Diabetes retinopathy are growing contagious disease , due to the current habits of consumption of energy-rich foods and lazyness about physical activity^[2]. Diabetic retinopathy is the leading cause of myopia in younger affecting over 90% patients with 20 years of diabetes. it is responsible for 4.8% of the 37million cases of eye disease related blindness worldwide^[3]. Diabetes increasing very rapidly, the number of patients with diabetic retinopathy is expected to grow from 126.6 million in 2010 to 191.0 million by 2030 ^[4]. This gradually progress disease is mainly indicate the damage of the retina and other part of eye. Instead of other changes of this slow progressing disease include hemorrhages, cotton wool spots, intra retinal microvascular abnormalities, and venous bleeding ^[5]. When diabetes increasd in upper stahe fragile vessels are formed along the retina and on the posterior surface of the vitreous, If this disease not diagnose at early stage they result in the detachment of the retina, leading to blindness.

This review is based on the epigenetic modification in diabetes retinopathy and their methylation at the 5HMC gene with the help of TeT protein and the HMC might be treat the Type II Diabetic Retinopathy in the BDNF gene^[12].

This review define the epigenetic modification in the BDNF gene at the specific region at 5HMC in the Type II Diabetic retinopathy.

This literature may be useful for diagnosing and also for therapeutic purpose because this review show the relation between the BDNF and the TypeII Diabetic retinopathy, BDNF mainly present in the brain and eyes . This study all about the epigenetic modification in BDNF gene specify the 5hmc region.

Collectively, these results indicate the essential roles of DNA methylation in both development and function of brain.

DNA Methylation

Recent study shows that hydroxylation of 5mC by ten-eleven translocation (TET) proteins leads to the formation of 5-hydroxymethylcytosine (5hmC), which can then mediate active DNA demethylation Tet methylcytosine dioxygenases (TET) convert 5-mC to 5-hmC^[22], 5-hydroxy methylcytosine, that can be further deaminated by AID/APOBEC, activation-induced cytidine deaminase/apolipo protein B mRNA editing enzyme catalytic polypeptide, to produce a DNA mismatch that is then repaired by the base excision repair machinery^[23].

Brain levels of tet methylcytosine dioxygenase 1 (TET1) were also found to correlate positively with *Bdnf* expression Similarity between DNA methylation in whole blood and regions of the brain was investigated The authors discovered distinct tissue-specific differences in DNA methylation, especially in genes involve neuronal development and differentiation, including an intronic region in BDNF. The tissue- specific variation was predominant in intragenic CpG islands^[24].

The three mammalian TET proteins, namely TET1, TET2 and TET3, are Fe²⁺-and 2-oxoglutarate-dependent dioxygenases that successively oxidize 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) in DNA. The activity of recombinant DNMT1 is reduced 12-fold⁵⁰ or 50-fold⁴⁹ at sites of hemi-5hmC in vitro^[25]. Together, these results imply that the TET-mediated hydroxyl methylation of amethylated CG site in vivo can block maintenance methylation during cell division and eliminate 5mC in a 'passive', replication-dependent manner^[25].

DNA methylation Method for the specific gene-

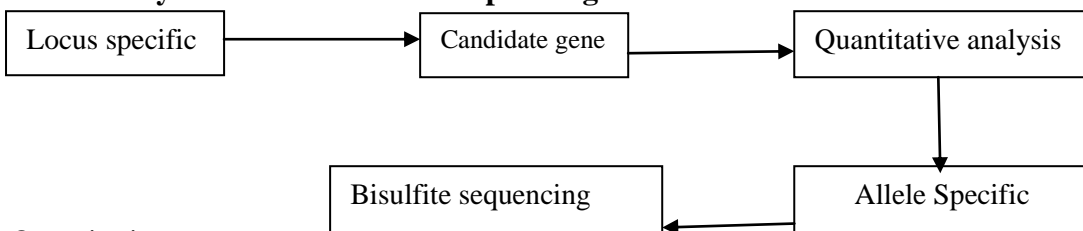


Figure 1: Methods of DNA methylation analysis by Lanlan Shena and Robert A. Waterland^[26]

3 Mechanism Of Dna Demethylation

DNA demethylation mechanism is found to be mediated by the ten–eleven translocation (Tet) enzymes Tet1, Tet2, and Tet3^[14]. Tet In the first, iterative oxidation by Tet enzymes continues to oxidize 5hmC first to 5-formyl-cytosine and then

to 5-carboxy-cytosine In the second, 5hmC is deaminated by AID/APOBEC to form 5-hydroxymethyl-Uracil Consistent with the role of Tet in converting 5mC into 5hmC in vivo^[13],

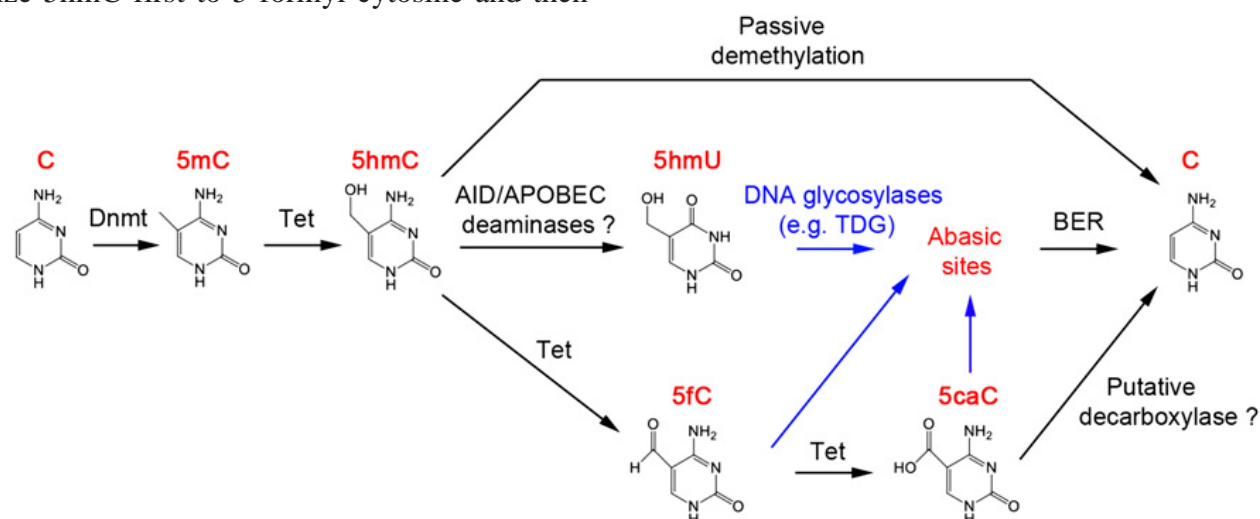


Figure 2: Mechanisms and functions of Tet protein mediated 5-methylcytosine oxidation Hao Wu^{1,2,3,4} and Yi Zhang^{1,2,5}

Proposed models of Tet-initiated DNA demethylation pathways. DNA methylation (5mC) is established and maintained by DNA methyltransferases (Dnmt)^[27]. In mammals, 5mC can be hydroxylated by the Tet family of dioxygenases to generate 5hmC. 5hmC is recognized poorly by Dnmt1 and can lead to replication- dependent passive demethylation. 5hmC can be further oxidized by Tet proteins to produce 5fC and 5caC^[28]. Alternatively, 5hmC may be further deaminated to become 5hmU by AID/APOBEC deaminases. 5hmU, 5fC, and 5caC can be excised from DNA by glycosylases such as TDG^[29]. A putative decarboxylase may directly convert 5caC to C. Cytosine and its

derivatives are highlighted in red. DNA glycosylase TDG-catalyzed reactions are indicated by blue arrows^[27].

Two replication-independent (‘active’) demethylation mechanisms have been reported to couple the methylcytosine oxidase activity of TET proteins with base excision repair (BER)^[25]. The first, which involves 5fC, 5caC and TDG, has been confirmed by multiple laboratories. The second mechanism, which involves AID (activation-induced cytidine deaminase) and APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide), is still controversial^[25]. In the first mechanism, TET proteins further oxidize 5hmC to generate 5fC and 5caC^{5,6,28}. 5fC and

5caC can be excised by TDG; their replacement with cytosine results in demethylation^[27].

This sequential deamination and removal of 5hmC is similar to the deamination of 5mC and the removal of the resulting T:G mismatches by TDG,

a mechanism previously proposed to occur in zebrafish^[25].

DNMT enzymes can remove the hydroxymethyl group of 5hmC in vitro, directly converting 5hmC to cytosine^[30].

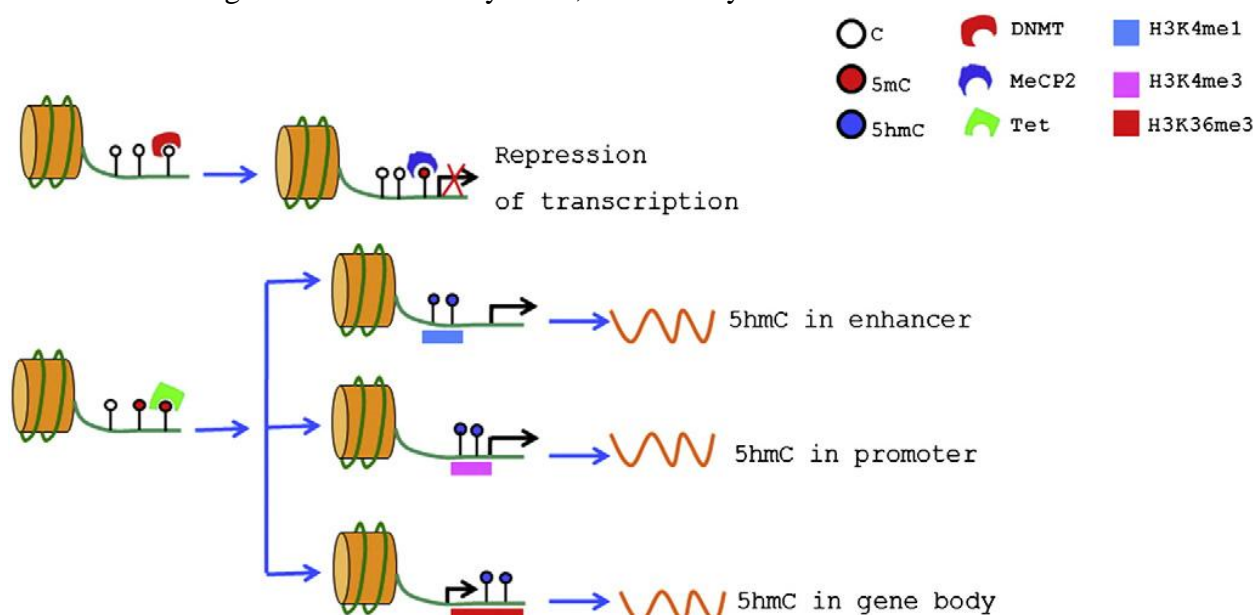


Figure 3: The schematic illustration of the relationship of DNA methylation and demethylation and gene expression. A. DNA methylation in promoter region leads to the repression of gene expression. B. Tet gene members convert 5mC to 5hmC in distinct genomic regions, which potentially involves in the regulation of gene expression^[27].

Detection of Hydroxymethyl Cytosine

Finally, we would like to briefly describe methods that aim to detect 5-hydroxymethylcytosine (5-hmC) and distinguish it from 5 mC. Essentially, there are two types of approaches for which this can be achieved: digestion- and antibody-based techniques^[31]. It has been determined that the enzyme glucosyltransferase will modify 5-hmC, but not 5 mC bases. Based on this intrinsic difference in their properties as substrates, both New England Bio labs and Zymo Research have developed assay kits for the specific detection of glycosylated 5-hmC. Zymo Research's Quest hmC Detection Kit utilizes hydroxymethylcytosine glucosyltransferase and the substrate uridine diphosphoglucose to selectively glucosylate 5-hmC bases^[31]. Importantly, modification of the 5-hmC bases renders these sites of the DNA strand resistant to cleavage by the nuclease MspI. Digested DNA is after that analyzed by both NGS or the qPCR assay. The EpiMark5-hmC and 5-mC

Analysis Kit by New England Bio labs uses the action of T4 beta-glucosyltransferase, followed by MspI digestion to accomplish the same task. Enrichment for 5-hmC-containing DNA could be done with anti-5-hmC antibodies. They are available from several companies^[31].

Conclusions and future directions

The serum BDNF levels were significantly higher in the T2DM patients compared to the healthy controls. BDNF has a central role in Diabetes Retinopathy. The rapid pace of research in the epigenetic modifications and mechanisms controlling BDNF gene expression indicates that progress in BDNF epigenetics will have wide spread applications in diagnosis, prognosis and bio markers for the Diabetes. Epigenetic mechanisms are hypothesized to play a role in the Diabetic retinopathy because they provide a mechanism for continued altered gene expression without the presence of the initiating HG stimulus

It has been reported that HG can induce site-specific DNA methylation. Present study concluded that level of DNA methylation in BDNF in retinal endothelial cells affect the microvasculature of human retina that can 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 II Diabetic Retinopathy and hints about their unique role in the pathogenesis of disease. Past studies have indicated that 5hmC does not merely serve as a DNA demethylation intermediate but also functions as a stable epigenetic mark. 5hmC enriches in gene bodies, promoters, and transcription factor binding sites and mounting evidence suggest roles of 5hmC in regulating gene expression and controlling cell identity. The dysregulation of 5hmC levels may lead to Diabetes Retinopathy and neurodegenerative diseases. These discoveries collectively provide new insights in understanding the function of this epigenetic modification including DNA demethylation in eyes. The current evidences mainly indicate a correlative relationship between 5hmC enrichment and gene expression. In order to allow better understanding the function of 5hmC, and finally it may be very helpful for therapeutic purpose in the Diabetes Retinopathy.

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