www.jmscr.igmpublication.org
Impact Factor (SJIF): 6.379
Index Copernicus Value: 71.58
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v6i5.109

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Pathogenesis of Type 2 Diabetic Retinopathy by the Epigenetic Modification of 5-Carboxylcytosine DNA Methylation in the CpG Island of Promoter Region of Brain Derived Neurotrophic Factor

Authors
Ambika Prasad Ahirwar[1], Rigved Tripathi[2], Pushpendra Kumar Namdeo[3], Archana Tiwari[4]
[1,2,3]Students,Department of Biotechnology, Rajiv Gandhi Proudyogiki Vishwavidyalaya
[4]Head of the Department, Department of Biotechnology Rajiv Gandhi Proudyogiki Vishwavidyalaya (State Technological University of Madhya Pradesh), Bhopal, Madhya Pradesh 462033 India.
Corresponding Author
Ambika Prasad Ahirwar
Email: *ambikabiotech111@gmail.com*, Mob: 8085957699

Abstract
Worldwide Type 2 Diabetic Retinopathy is one of the leading causes of blindness. It is the most frequently occurring complication of diabetes mellitus and remains a leading cause of vision loss in many countries. Although some potential functions of DNA methylation have been demonstrated already, many questions remain in terms of unveiling the role of 5caC; whether it serves either merely as an intermediate of DNA demethylation or as a stable epigenetic marker. 5-carboxylcytosine (5caC) is proved to be not merely serving as an intermediate of DNA demethylation, but also acts as a stable epigenetic marker. This review define how to control the gene expression and DNA Methylation in the CpGIsl and, The DNA Demethylation leads to convert the 5mc to 5hmc by the TeTprotein and again the 5hmc transfer to 5fc and further 5caC by the TeT enzyme in the BDNF gene. This review show the method to analyze the DNA Methylation at the 5-caC region for the BDNF gene and the fully mechanism which show the demethylation from 5hmc to 5fc and the pathway of DNA Demethylation from cytosine to 5-caC (5-Carboxylcytosine).
Keywords: *Epigenetic Modification, DNA methylation, BDNF gene, 5-caC, CpGIs , Diabetic Retinopathy.*

Introduction

Pathology of diabetic retinopathy has been studied for half a century, yet there are disappointingly few therapeutic options. Though some new treatments have been made known for Diabetic Macular Edema (DME) (e.g. anti-VEGFs' (intravitreal vascular endothelial growth factor inhibitors) and other novel steroids), only up to 50% of patients could respond. Laser photocoagulation remains a mainstay therapy (even though it is inherently a destructive procedure)for people with proliferative diabetic retinopathy (PDR).[1] In many diseases epigenetics is now emerging as one of the important factors as it can regulate the complex interplay between genes and the environment, and these heritable changes can occur without any change in the DNA sequence.Influenced by several factors

including age, environment, lifestyle and disease state epigenetic changes are regular and natural phenomenon. Epigenetic changes can work like switches helping to control gene activity and allowing alternations in genome function without altering the gene sequences. DNA methylation, histone modification and non-coding RNA is considered to initiate and sustain changes in gene regulation are major epigenetic modifications. [2]

Epigenetic mechanisms in the pathogenesis of type 2 diabetes

Methylation of cytosine to 5-methyl cytosine (5mC) 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 interferes with the binding of transcriptional machinery, resulting in gene suppression.[3] DNA methylation is catalyzed by DNA methyl transferases (Dnmts), a family with five members- Dnmt1, Dnmt2, Dnmt3a, Dnmt3b and Dnmt3L; out of which only Dnmt1, Dnmt3a and Dnmt3b are catalytically active, and Dnmt3b are de novo methyltransferases, and Dnmt1 is a maintenance enzyme important in regulating tissue-specific patterns of methylated cytosine residues.[4]

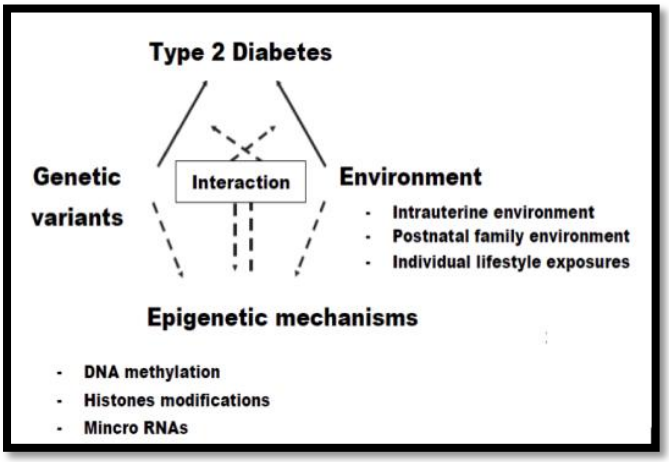

Figure 1. Role of epigenetic mechanisms in the pathogenesis of type 2 diabetes proposed by model.[5]

DNA Demethylation Pathways

By a reduction in activity or absence of DNA methyltransferases (Dnmts) (black) Passive DNA demethylation has long been known to occur.For de novo DNA methylation Dnmt3A and 3B are responsible. Whereas Dnmt1 maintains DNA methylation patterns through successive rounds of cell division. Three enzyme families have been implicated in active DNA demethylation via DNA repair recently.

1. Ten eleven translocation (TET) family of enzymes (blue) can hydroxylate 5-methylcytosine (5mC) to form 5-hydroxymethylcytosine (5hmC) or further oxidized to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC).
2. AID/APOBEC (Activation-induced Cytidine Deaminase /apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) family members (purple) can deaminate 5mC (or 5hmC) to form 5-methyluracil (5mU) or 5-hydroxymethyluracil (5hmU)
3. Uracil-DNA Glycosylase (UDG) family of base excision repair (BER) glycosylases (green) initiate replacement of these intermediates (i.e., 5mU, 5hmU, or 5caC) like TDG or SMUG1, culminating in cytosine replacement and DNA demethylation.[6]

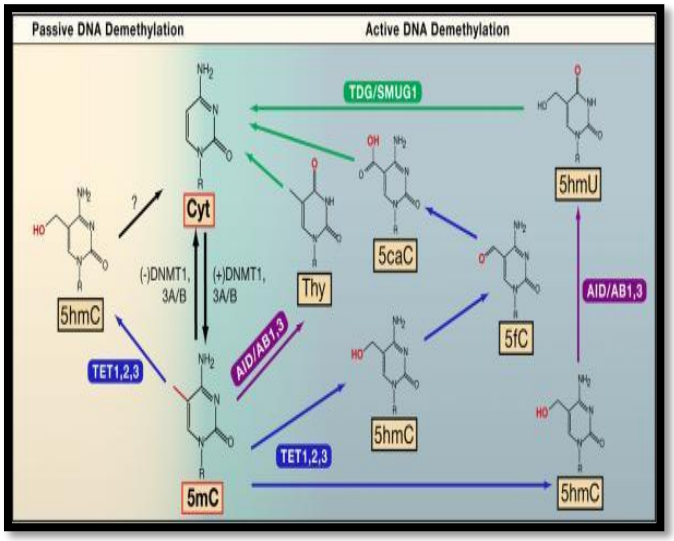

Figure 2: 5-caC methylation pathway.[6]

With neuro degeneration pathological Dnmt activity and aberrant 5mC formation have been linked. 5mC can be oxidized to 5-hydroxymethylcytosine (5hmC) by ten-eleven translocation enzymes, and 5hm Can also be further oxidized to generate 5-formylcytosine (5fc) and 5-carboxylcytosine (5caC).By removing the 5-methylcytosine base a subfamily of DNA glycosylases are assumed to promote active DNA demethylation, followed by cleavage of the DNA backbone at the abasic site, and an unmethylated cytosine can replace methylated cytosine. In contrast, the passive process involves absence/ inactivation of Dnmt1 resulting hypomethylated DNA.[7]

Epigenetic modifications (DNA methylation) and diabetes

Glucose is critically important for the organisms for survival, but sustained levels of high glucose are detrimental, and can initiate a number of metabolic, biochemical and genetic abnormalities. It affects regulation of genes throughout the body. Recent work has suggested that diabetic milieu favors epigenetic modifications in various organs associated with micro- and macro-vascular complication[8] S-Adenosyl methionine (SAM), the donor of methyl group for DNA methylation, is shown to influence the expression of genes related with diabetic complications, and blood deficiency of SAM is reported in the patients with diabetic nephropathy, and leukocytes from diabetic patients have reduced Dnmtslevels. Globalhypomethylation and reduced level of SAM are also observed in Zebrafish with chemically induced diabetes.[9] In contrast, in Zucker fatty rat, a model of Type 2 diabetes, sustained global DNA hypermethylation is observed in the liver, and this is associated with abnormal metabolism of the methyl group. These divergent patterns of DNA methylation in diabetes suggest that various tissues could be responding differently to diabetes.[10-11]

Epigenetic modification in diabetic retinopathy

Diabetic retinopathy, a slow progressing disease, is associated with a number of metabolic abnormalities. However, the role of epigenetic modifications in diabetic retinopathy is still not clear. In a Finnish study, an association between the polymorphism in SUV39H2, a gene that encodes histone methyltransferases and micro-vascular complications, including retinopathy has been observed in patients with diabetes. In a cross-sectional study with over 1,000 patients having type 2 diabetes, analysis of their family history has suggested a possible genetic and epigenetic basis for the development of diabetic retinopathy. laboratory proof using in vitro and in vivo models of diabetic retinopathy have shown that the actions of HDACs are increased and that of HATs are decline in the retina and its capillary cells in diabetes, and global acetylating of histones is decreased.[12-13] DNA methylation, an important epigenetic modification, is closely associated with the regulation of gene transcription. A case control study using patients having type 2 diabetes has shown significantly higher levels of global DNA methylation in patients having diabetes with retinopathy compared to those with no retinopathy, and although global DNA methylation appears to be independent of retinopathy risk factors, e.g., hyperglycemia, dyslipidemia and hypertension, in these patients, the methylation status of DNA shows a correlation with the progression of retinopathy.[14-15]

Type 2 Diabetic Retinopathy

Type 2 Diabetic Retinopathy is 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 Type 2Diabetic Retinopathy. 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 ways: Macula Edema

and Proliferative retinopathy.[16-17] Type 2 Diabetic Retinopathy classified into two clinical forms they are non proliferative diabetic retinopathy, proliferative diabetic retinopathy. Nonproliferative diabetic retinopathy subdivided into 3 different stages they are mild, moderate and severe.[18-19] Nonproliferative diabetic retinopathy is being characterized by the quantity of microaneurysms, dot and blot hemorrhages, cotton wool spots and blood vessel abnormalities.[20] 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.[20]In case of proliferative diabetic retinopathy improvement of glaucoma, retinal detachment, and vision loss may additionally manifest.[21]

5. Ocular complications of - diabetes Diabetic retinopathy

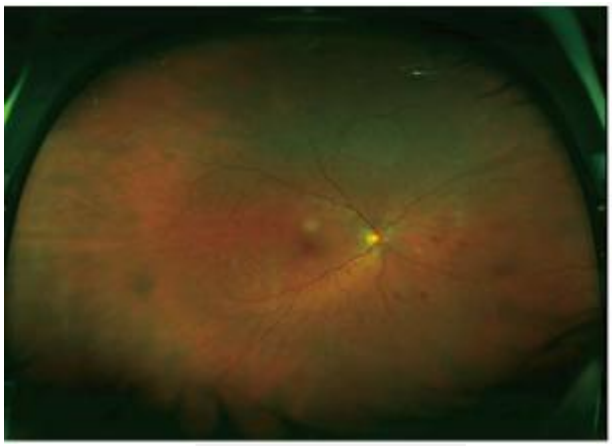

Figure 3.A

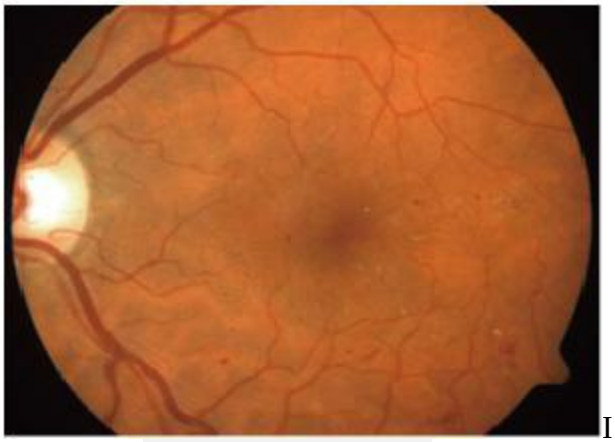

Figure 3.B

Figure 3 (A) Non-proliferative diabetic retinopathy. Wide-field fundus photo of 65-year-oldfemale patient (right eye) showing several retinal hemorrhages

Figure 3 (B) Non-proliferative diabetic retinopathy. Color fundus photo of a 51-year-old male patient with micro-aneurysms and lipid exudates.[22]

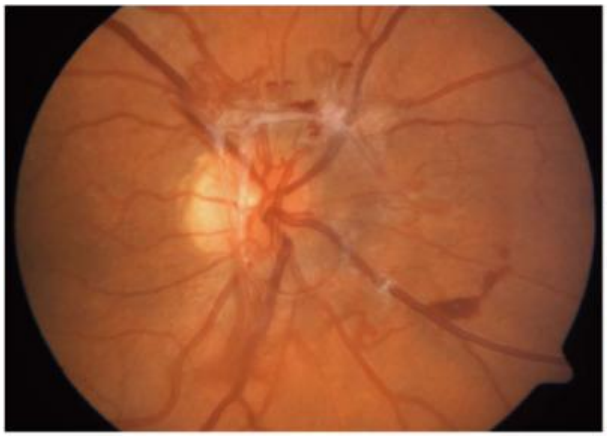

Figure 4.A

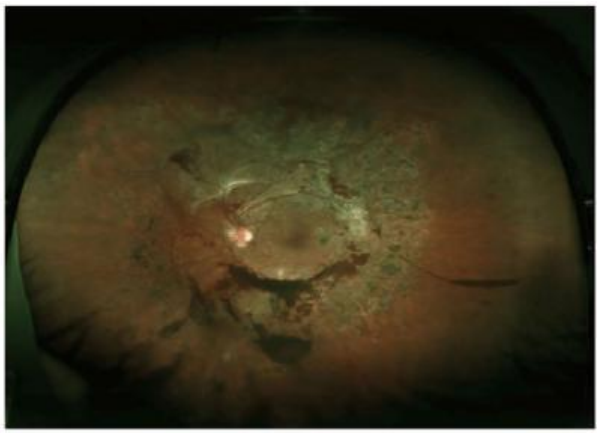

Figure 4.B

Figure 4 (A) Proliferative diabetic retinopathy with neovascularization at disk.

Figure 4 (B) Advanced proliferative diabetic retinopathy with neovascularization and limited vitreous hemorrhage.[22]

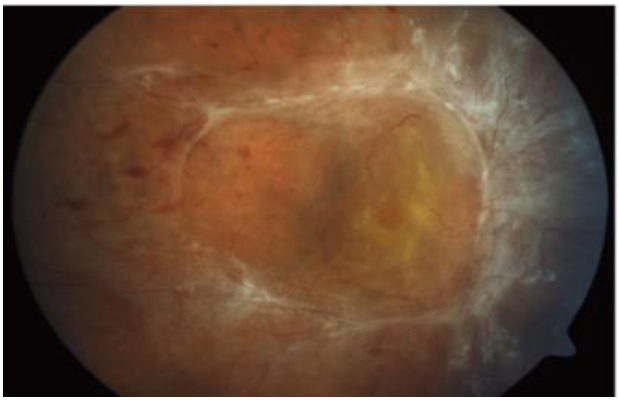

Figure 5.A

Figure 5 (A) Advanced proliferative diabetic retinopathy with tractional retinal detachment.

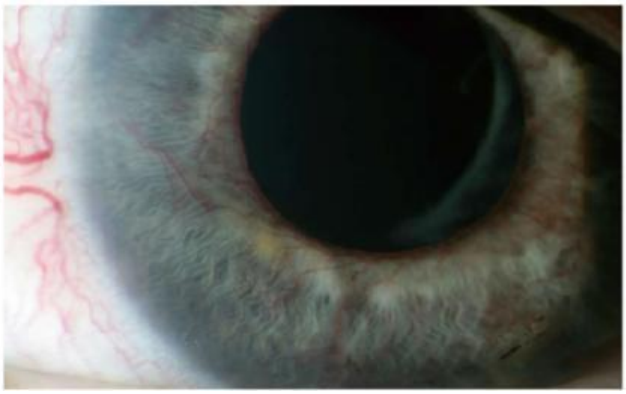

Figure 5.B

Figure 5 (B) Neovascularization of the iris. These neovascular vessels may block the trabecular meshwork and cause neovascular glaucoma.[22]

BDNF (Brain Derived Neurotrophic Factor)

Nerve growth factor (NGF) was discovered in the early 1950s due to its trophic (survival- and growth-promoting) effects on sensory and sympathetic neurons. To support survival of a subpopulation of dorsal root ganglion neurons, and subsequently purified from pig brain, in 1992, Brain-derived neurotrophic factor (BDNF), the second member of the "neurotrophic" family of neurotrophic factors, was demonstrated.[23] Since then, other members of the neurotrophin family such as neurotrophin-3 (NT-3)[24] and neurotrophin-4/5 (NT-4/5)[25-26] have been described, each with a distinct profile of trophic effects on subpopulations of neurons in the peripheral and central nervous system.[26]

BDNF gene structure

The human BDNF gene is located on chromosome 11, region p13– 14 and it spans ~ 70 kb. The gene has a complex structure as it consists of 11 exons (I–IX, plus Vh and VIIIh) in the 5′ end and of nine functional promoters that are used in tissue and brain regions specifically, namely exon I, II (with the transcripts IIa, IIb and IIc), III, IV, V (with the transcripts Va, Vb, Vc and V–VIII–VIIIh), VI (with the transcripts VIb, VIb–IXabd and VIb–IXbd), VII (with transcripts VIIa and VIIb) and IX (with transcripts IXabd and IXabcd; Figure 6).[27]

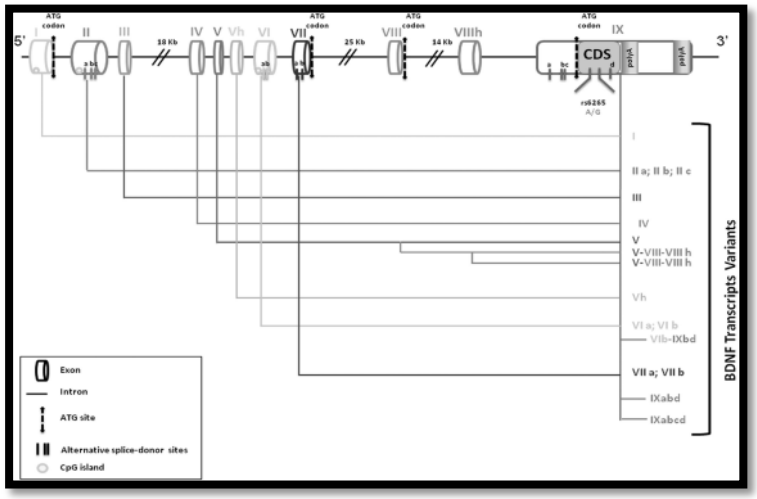

Fig. 6

Human BDNF gene structure. In the figure the structure of the human BDNF gene is shown; the gene contains 11 exons (I–IX, plus Vh and VIIIh), which combine to many different transcripts. In exon IX hasCoding DNA sequence (CDS). CpG islands and Alternative splice-donor sites are also reported BDNF. [27]Although the BDNF gene comprises nine exons, the coding sequence resides in exon 9, with eight upstream exons that encode promoters regulating regional and cell-type-specific expression. [27] Among these, exon IV has been the most extensively characterized, as this exon, containing promoter elements, regulates activity-dependent BDNF expression. [27]

BDNF and Diabetic Retinopathy

In the animal model with Type 2 Diabetic Retinopathy, few studies suggest that reduced level of BDNF in the Type 2 Diabetic Retinopathy may damage neurons, thereby leading to neuro degeneration. [28] Another study reported that through endothelial progenitor cells activation neuroic factors may induce neoangiogenesis, leading to the pathological retinal neovascularisation found that BDNF was effective in protecting retinal neurons from hyperglycemia in vitro. The patients with Type 2 Diabetic Retinopathy were found to have a drastic reduction in the level of BDNF in retina.[29]If any alteration occurs in any signaling 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 Type 2 Diabetes mellitus compared to non-diabetic individuals, both in plasma and serum, and in different populations.[30]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 patho physiological process of insulin resistance and Type 2 Diabetes which may cause Type 2 Diabetic Retinopathy.[31]

5-caC (5-Carboxylcytosine) DNA methylation

When Tet enzymes oxidize 5-methylcytosine, 5-hydroxymethylcytosine and subsequently 5-formylcytosine5- Carboxylcytosine has been identified as one of the DNA methylation variants. It is believed that the oxidation of 5-methylcytosine through to 5-carboxylcytosine represents a mechanism of DNA demethylation, and that this demethylation pathway has a function during development and germ cell programming[32]. It has been suggested that 5-caC is excised from genomic DNA by thymine DNA glycosylase (TDG), which returns the cytosine residue back to its unmodified state. 5-Carboxylcytosine has been identified in mouse embryonic stem (ES) cells.[32-33] This DNA modification appears in the paternal pronucleus post-fertilization, concomitant with the disappearance of 5-methylcytosine, further lending support that this variant is part of a DNA demethylation pathway. Unmodified DNA standard and 5-carboxylcytosine DNA standard, which can be used as negative and positive controls, respectively, in the analysis of 5-carboxylcytosine.[34]

Detection of 5'caC (5-Carboxylcytosine)

MAB (methylation-assisted bisulfite sequencing) Method Enables Highly Efficient Detection of 5caC.

(A) Whereas the 5-methyl and 5-hydroxymethyl cytosine are protected Bisulfite treatment of DNA leads to deamination of cytosine, 5fC, and 5caC. Treatment of DNA with the S-Adenosyl-methionine- dependent CpG methyltransferase M.SssI prior to bisulfite treatment leads to the conversion of unmodified cytosines to 5-methyl cytosine, thus enabling the specific detection of 5fC and 5caC residues.(B) Containing only unmodified, 5-methyl, 5- hydroxymethyl, 5-formyl, or 5-carboxyl cytosine residues, Validation of MAB on five DNA standards. Dot blot analysis reveals complete conversion of unmodified cytosines to 5-methyl cytosines following M.SssI treatment. After bisulfate treatment, Sanger sequencing detects 5fC and 5caC as T, whereas unmodified C (methylated by M.SssI), 5mC, and 5hmC are detected as.[35]

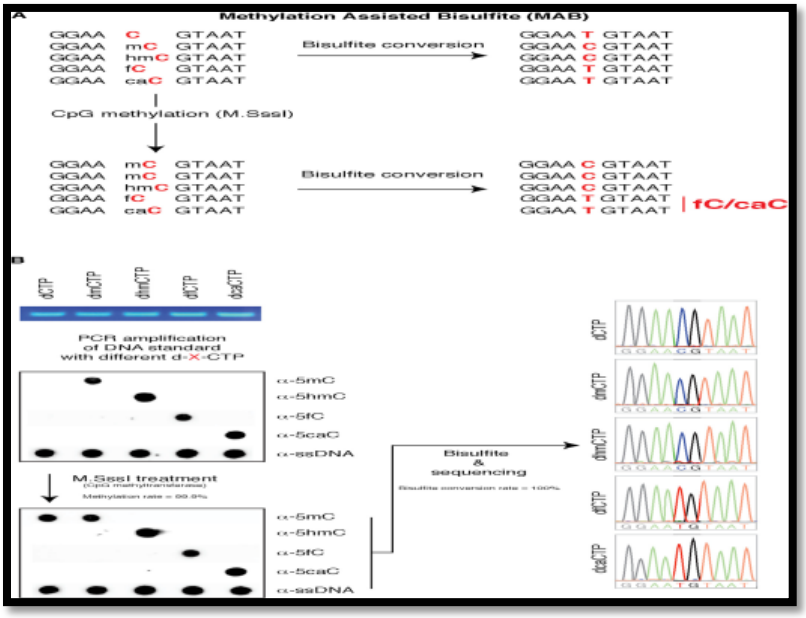

Figure 7. MAB Method Enables Highly Efficient detection of 5caC (A) Bisulfite treatment (B) Validation of MAB [35]

7. DNA Methylation Analysis

By adding methyl or hydroxymethyl group to the C5 position of cytosine, DNA methylationin vertebrates was characterized, which occurs mainly in the context of CG dinucleotides. Non-CpG methylation in a CHH and CHG context (where H = A, C or T) exist in embryonic stem cells.[36]

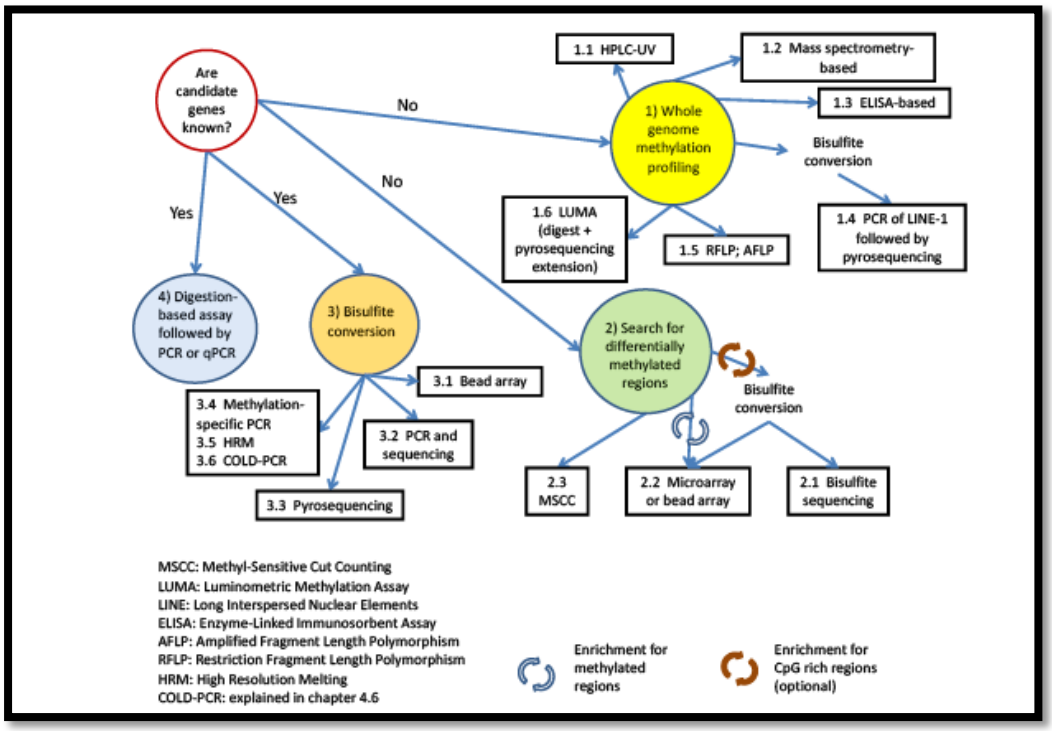

Figure 8.A graphical guide for choosing the right method for a specific project usinga simple algorithm.[36]

Serum Level of BDNF in Diabetic and PDR Patients

Measured systemic level of BDNF in the serum of nondiabetic, diabetic with no retinopathy, and PDR patients. The mean serum levels of BDNF in diabetic patients and PDR patients were 21.8 ± 4.9 and 10.01 ± 8.1 ng/ml, respectively (Fig. 9**)**. In the serum of control patients,the mean BDNF level was 25.5 ± 8.5 ng/ml. Statistical analysis suggests that in PDR patients, the serum level of BDNF is significantly reduced (p\0.001) compared to that of non-diabetic controls and diabetic patients (ANOVA). The level of BDNF in diabetic serum was modestly reduced to 15 % compared to that in nondiabetic control; however, the difference was not significant (p = 0.38).[37]Serum levels of BDNF in control, diabetic, and PDR patients were measured by ELISA kit. To the level from non-diabetic controls (n = 19) and early diabetic patients (n = 22) Reduced levels of BDNF in PDR groups (n = 47) were compared. Mean non-diabetic BDNF (25.5 ± 8.5 ng/ml), diabetic (21.8 ± 4.7 ng/ml), and PDR (10.0 ± 8.1 ng/ml). Mean values were compared across groups by one-way ANOVA and post hoc test for multiple comparisons. Values are mean ± SD, *#p\0.001 compared with control and diabetic.[37]

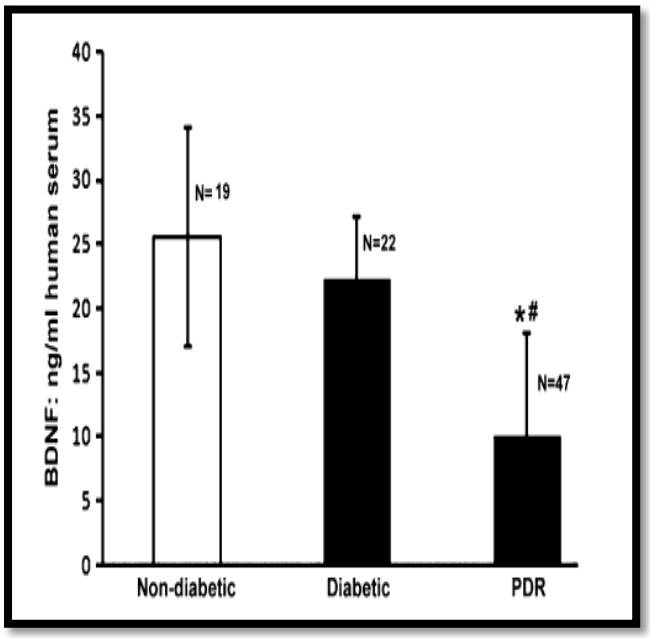

Figure 9. BDNF serum concentration in healthy controls, diabetic patients, and PDR patients.[37]

Conclusions

Epigenetic mechanisms 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 noted that hyperglycemia can induce site-specific DNA methylation. This 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. The serum BDNF levels were significantly higher in the Type 2 Diabetic mellitus patients compared to the healthy controls. BDNF has a central role in Diabetic 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. This is hypothesized that in the Diabetic retinopathy Epigenetic mechanisms play a role because they provide a mechanism for continued altered gene expression without the presence of the initiating HG(H=A,C,T) 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 2 Diabetic Retinopathy and hints about their unique role in the pathogenesis of disease. Previous studies suggested that 5caC merely serve as a DNA demethylation intermediate but functions as a stable epigenetic mark. 5caC 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 novel insights in understanding the function of this epigenetic modification including DNA demethylation in eyes. The current evidences mainly indicate a correlative relationship between 5caC enrichment and gene expression. With a view to allow better understanding the function of 5cac finally it may be very helpful for therapeutic purpose in the Diabetes Retinopathy.

References

1. Stitt, A. W., Curtis, T. M., Chen, M., Medina, R. J., McKay, G. J., Jenkins, A., & Lois, N. (2016). The progress in understanding and treatment of diabetic retinopathy. *Progress in Retinal and eye Research*, *51*, 156-186.
2. Barros, S. P., & Offenbacher, S. (2009). Epigenetics: connecting environment and genotype to phenotype and disease *Journal of dental research*, *88*(5), 400-408.
3. Rose, N. R., & Klose, R. J. (2014). Understanding the relationship between DNA methylation and histone lysine methylation. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, *1839*(12), 1362-1372.
4. Lyko,F.(2017).TheDNA methyltransferase family: A versatile toolkit for epigenetic regulation. *Nature Reviews Genetics*, nrg-2017.
5. Gouri, A., &Dekaken, A. (2013). pathways in type 2 diabetes and its complications. *ANNALS*, *1*(1), 1
6. Bhutani, N., Burns, D. M., &Blau, H. M. (2011). DNA demethylation dynamics. *Cell*, *146*(6), 866-872.
7. Zhang, P. (2016). The regulation of Ten-eleven translocation proteins (methylcytosine modifiers) by methyl-CpG binding domain proteins (methylcytosine readers) (Doctoral dissertation, Technische Universität).
8. Kato M, Natarajan R. Diabetic nephropathy—emerging epigenetic mechanisms. Nat Rev Nephrol, 2014, 10: 517–530
9. Tewari S, Zhong Q, Santos JM, Kowluru RA. Mitochondria DNA replication and DNA methylation in the metabolic memory associated with continued progression of diabetic retinopathy. InvesOphthalmol Vis Sci, 2012, 53: 4881–4888
10. Williams KT, Garrow TA, Schalinske KL. Type I diabetes leads to tissue-specific DNA hypomethylation in male rats. J Nutr, 2008, 138: 2064–2069
11. Williams KT, Schalinske KL. Tissue-specific alterations of methyl group metabolism with DNA hypermethylation in the Zucker (type 2) diabetic fatty rat. Diabetes Metab Res Rev, 2012, 28: 123–131
12. Zhong Q, Kowluru RA. Role of histone acetylation in the development of diabetic retinopathy and the metabolic memory phenomenon. J Cell Biochem, 2010, 110: 1306–1313
13. Kadiyala CS, Zheng L, Du Y, Yohannes E, Kao HY, Miyagi M, Kern TS. Acetylation of retinal histones in diabetes increases inflammatory proteins: effects of minocycline and manipulation of histone acetyltransferase (HAT) and histone deacetylase (HDAC). J Biol Chem, 2012, 287: 25869–25880
14. Maghbooli Z, Hossein-Nezhad A, Larijani B, Amini M, Keshtkar A. Global DNA methylation as a possible biomarker for diabetic retinopathy. Diabetes Metab Res Rev, 2015, 31: 183–189
15. Maghbooli Z, Larijani B, Emamgholipour S, AminiM, Keshtkar A, Pasalar P.

Aberrant DNA methylation patterns in diabetic nephropathy. J Diabetes MetabDisord, 2014, 13: 69
16. Frank RN. Diabetic retinopathy. N Engl J Med, 2004;350:48– 58.
17. May, S. E. (2014). An Investigation of Diabetes Mellitus in Postmortem Human Remains.
18. Lai CM, Dunlop SA, May LA, Gorbatov M, Brankov M, Shen WY, Binz N, Lai YK, Graham CE, Barry CJ, Constable IJ. Generation of transgenic mice with mild and severe retinal neovascularization. British journal of ophthalmology. 2005 Jul 1;89(7):911-6.
19. Yilmaz, T., & Yilmaz, A. (2016). Relationship between altered platelet morphological parameters and retinopathy in patients with type 2 diabetes mellitus. Journal of ophthalmology, 2016.
20. Curtis, T. M., Gardiner, T. A., & Stitt, A. W. (2009). Microvascular lesions of diabetic retinopathy: clues towards understanding pathogenesis?E3(ye, 27), 1496.
21. Robinson R, Barathi VA, Chaurasia SS, Wong TY, Kern TS. Update on animal models of diabetic retinopathy: from molecular approaches to mice and higher mammals. Disease models & mechanisms. 2012 Jul 1;5(4):444-56.
22. Nentwich, M. M., &Ulbig, M. W. (2015). Diabetic retinopathy-ocular complications of diabetes mellitus. World journal of diabetes, 6(3), 489
23. Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. EMBO J 1982;1:549–553.
24. Maisonpierre PC, et al. Neurotrophin-3: a neurotrophic factor related to NGF and BDNF. Science 1990;247:1446–1451.
25. Hallbook F, Ibanez CF, Persson H. Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in Xenopus ovary. Neuron 1991;6:845–858.
26. Ip NY, et al. Mammalian neurotrophin-4: structure, chromosomal localization, tissue distribution, and receptor specificity. Proc Natl Acad Sci USA 1992;89:3060–3064.
27. Cattaneo, A., Cattane, N., Begni, V., Pariante, C. M., & Riva, M. A. (2016). The human BDNF gene: peripheral gene expression and protein levels as biomarkersfor psychiatric disorders. Translational psychiatry, 6(11), e958.
28. Nguyen, L., Lucke-Wold, B. P., Mookerjee, S. A., Cavendish, J. Z., Robson, M. J., Scandinaro, A. L., & Matsumoto, R. R. (2015). Role of sigma-1 receptors in neurodegenerative diseases. Journal of pharmacological sciences, 127(1), 17-29.
29. Mysona, B. A., Shanab, A. Y., Elshaer, S. L., & El-Remessy, A. B. (2014). Nerve growth factor in diabetic retinopathy: beyond neurons. Expert review of ophthalmology, 9(2), 99-107.
30. Menezes Zanoveli, J., de Morais, H., Caroline da Silva Dias, I., Karoline Schreiber, A., Pasquini de Souza, C., & Maria da Cunha, J. (2016). Depression associated with diabetes: from pathophysiology to treatment. Current diabetes reviews, 12(3), 165-178.
31. Rani, J., Mittal, I., Pramanik, A., Singh, N., Dube, N., Sharma, S., ... & Ramachandran, S. (2017). T2DiACoD: A Gene Atlas of Type 2 Diabetes Mellitus Associated Complex Disorders. Scientific reports, 7(1), 6892.
32. He, Y. F., Li, B. Z., Li, Z., Liu, P., Wang, Y., Tang, Q., ... & Sun, Y. (2011). Tet-mediated formation of5-carboxylcytosine and its excision by TDG in mammalian DNA. Science, 333(6047), 1303-1307.
33. Ito, S., Shen, L., Dai, Q., Wu, S. C., Collins, L. B., Swenberg, J. A., ... & Zhang, Y. (2011). Tet proteins can convert

5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science*, *333*(6047), 1300-1303.

34. Inoue, A., Shen, L., Dai, Q., He, C., & Zhang, Y. (2011). Generation and replication-dependent dilution of 5fC and 5caC during mouse preimplantation development. Cell research, 21(12), 1670.
35. Neri, F., Incarnato, D., Krepelova, A., Rapelli, S., Anselmi, F., Parlato, C., ... & Oliviero, S. (2015). Single-base resolution analysis of 5-formyl and 5-carboxyl cytosine reveals promoter DNA methylation dynamics. Cell reports, 10(5), 674-683.
36. Kurdyukov, S., & Bullock, M. (2016). DNA methylation analysis: choosing the right method. Biology, 5(1), 3.
37. Ola, M. S., Nawaz, M. I., El-Asrar, A. A., Abouammoh, M., &Alhomida, A. S. (2013). Reduced levels of brain derived neurotrophic factor (BDNF) in the serum of diabetic retinopathy patients and in the retina of diabetic rats. Cellular and molecular neurobiology, 33(3), 359-367.