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Epigenetic regulation of 5Hydroxy methyl cytosine region in the CpG Island of Brain derived neurotropic factor in human type II diabetes Retinopathy: An *in silico* approach

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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 research define how to control the gene expression and DNA Methylation in the CpG Island ,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 research show the method to analyze the DNA Methylation at the 5hmc 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 5CaC (Carboxyl acetyl cytosine).

Keywords: Epigenetic Modification, DNA Methylation,Brain Derived Neurotrophic Factor,TeT Protein.

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

In the present study, methylation activity of TET 2 enzyme was inhibited with suitable methylation inhibitors which led to the decrease of the hypermethylation of BDNF protein. The interaction study of this modified TET2 with BDNF alpha leads to the new way of drug discovery in type 2 diabetes.

Introduction-

Epigenetics is define as the reading of genetic alterations in gene appearance (active versus inactive genes) that does not include variations to the novel DNA sequence an alteration in phenotype without a modification in genotype which in turn disturbs how cells read the genes(Mostafalou S., 2013).Epigenetic alterations are genetic variations in gene expression not programmed by the DNA sequence. Epigenetic change is a regular and

natural occurrence but can also be influenced by several factors including age, the environment/lifestyle, and disease state(Qin Z ., 2012). At least three structures containing DNA methylation, histone modification and non-coding RNA (ncRNA)-related gene silencing are presently measured to start and sustain epigenetic alteration(McFall-Ngai et al .,2013).

Gene appearance can be regulate through the action of repressor proteins that link to silencer areas of the DNA(Qin Z ., 2012). Genetic data passes from generation to generation, a procedure called epigenetic tradition.Diabetic retinopathy, similarly identified as diabetic eye syndrome, is a medical disorder in which impairment occurs to the retina due to diabetes and is a important reason of vision loss. Diabetic retinopathy is the greatest frequent reason of new cases of sightlessness between adults aged 20 –74 years(Ezquer et al., 2016).Diabetic retinopathy developments from slight non proliferative irregularities, categorized by amplified vascular permeability, to reasonable and severe non proliferative diabetic retinopathy (NPDR), categorized by vascular end, to proliferative diabetic retinopathy (PDR), considered by the development of original blood vessels on the retina and subsequent surface of the vitreous(Maulucci et al., 2016).

Diabetic retinopathy is the result of damage to the small blood vessels and neurons of the retina. The earliest changes detected in the retina in diabetes leading to diabetic retinopathy include a narrowing of the retinal arteries associated with reduced retinal blood flow; dysfunction of the neurons of the inner retina, followed in later stages by changes in the function of the outer retina, associated with subtle changes in visual function; dysfunction of the blood-retinal barrier, which protects the retina from many substances in the blood (including toxins and immune cells), leading to the leaking of blood constituents into the retinal neuropile(Chisada et al., 2017).

Diabetic retinopathy too disturbs microcirculation thorough the body. A current study presented assessment of conjunctival microvascular hemodynamics such as vessel diameter, red blood cell velocity and wall shear stress can be useful for diagnosis and screening of diabetic retinopathy(Thamsen et al., 2015).Type 2 diabetes mellitus (T2DM) is a polygenic metabolic disease characterized by elevated blood sugar levels due to pancreatic beta-cell functional impairment and insulin resistance in tissues such as skeletal muscle, adipose tissue and the liver(Frank et al., 2010).Epigenetic is the study of heritable changes in gene function without any change in the nucleotide sequence. The human body's development, progress and digestion are carefully caused by the epigenetic mechanisms, which affect chromatin structure and DNA accessibility, leading to switching 'on' or 'off' our genes at strategic times and locations(Mostafalou et al., 2013).The famous epigenetic mechanisms that can change gene expression are DNA methylation, histone modification, and non-coding RNA-mediated pathways . Briefly, in DNA methylation, a methyl group is added at the 5-carbon of the cytosine to form 5-methylcytosine. DNA methylation generally results in gene silencing or reduced gene expression (Bading et al., 2013).

DNA Methylation-DNA methylation is an epigenetic mechanism that occurs by the addition of a methyl (CH_3) group to DNA, thereby often modifying the function of the genes and affecting gene expression. The most widely characterized DNA methylation process is the covalent addition of the methyl group at the 5-carbon of the cytosine ring resulting in 5-methylcytosine (5-mC), also informally known as the "fifth base" of DNA. These

methyl groups project into the major groove of DNA and inhibit transcription(Subramaniam et al., 2014).

A current definition of epigenetics is: "The study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence

(Björkegren et al., 2015).methylation was believed to play a crucial role in repressing gene expression, perhaps by blocking the promoters at which activating transcription factors should bind.proper DNA methylation is essential for cell differentiation and embryonic development.in some cases, methylation has observed to play a role in mediating gene expression. Evidence of this has been found in studies that show that methylation near gene promoters varies considerably depending on cell type, with more methylation of promoters correlating with low or no transcription(Smith et al., 2013).CpG islands are usually defined as regions with 1) a length greater than 200bp, 2) a G+C content greater than 50%, 3) a ratio of observed to expected CpG greater than 0.6, around 60-70% of human genes have a CpG island in their promoter region(Deaton et al., 2011).The finding of Ten-eleven translocation (TET) enzymes was one of the main new finding in epigenetics. They provided a mechanistic basis for a mostly hypothetical pathway, active DNA demethylation. The enzymes are named for a common translocation in cancers. A translocation can occur between chromosomes 10 and 11 creating a MLL-TET1 fusion protein(Lorsbach et al., 2003). 5-Hydroxymethylcytosine is a DNA pyrimidine nitrogen base resultant from cytosine. It is potentially significant in epigenetics, since the hydroxymethyl collection on the cytosine can maybe switch a gene on and off. It was first seen in bacteriophages in 1952.Its molecular formula is $C_5H_7N_3O_2$(Calo et al., 2013).The BDNF gene (in humans mapped to chromosome 11p) has four 5′ exons (exons I-IV) thatare related with different promoters, and one 3′ exon (exon V) that converts the developed BDNF protein (Metsis et al., 1993; Timmusk et al., 1993).BDNF acts on certain neurons of the central nervous system and the peripheral nervous system, helping to support the survival of existing neurons, and encourage the growth and differentiation of new neurons and synapses. In the brain, it is lively in the hippocampus, cortex, and basal forebrain parts energetic to learning, memory, and higher thinking. It is also expressed in the retina, motor neurons, the kidneys, saliva, and the prostate(Guy et al., 2011).Sequencing method is used to determine the pattern of methylation. Treatment of DNA with bisulfate (salt) con-verts cytosine residues to uracil, but leaves 5-methyl-cytosine residues unaffected. Thus, bisulfite treatment introduces specific changes in the DNA sequence that depend on the methylation status of individual cytosine residues, yielding single-nucleotide resolution information about the methylation status of a segment of DNA. Various analyses can be performed on the altered sequence to retrieve this information(Long-Cheng and Rajvir, 2002).

The present study investigated: (1) DNA methylation analysis of BDNF ; (2) the modification of the methy-lation activity of TET2 by methylation inhibitors.

MATERIALS AND METHODS

Protein Structure

The Structure of the enzyme TET2 and the BDNF protein was analyzed by SAVS server. the results are shown in Figure1(a)and1(b).

DNA methylation analysis

DNA methylation involves the addition of a methyl group to the 5TH position of the cytosine pyrimidine ring or the number 6 nitrogen of the adenine purine ring. The process takes place in the so called CpG islands, located in the promoter of the eukaryotic genes. The methylation

analysis was done using MethPrimer software. Meth-Primer software uses bisulfite sequencing method to determine the pattern of methylation. Treatment of DNA with bisulfate (salt) con-verts cytosine residues to uracil, but leaves 5-methylcytosine residues unaffected. Thus, bisulfite treatment introduces specific changes in the DNA sequence that depend on the methylation status of individual cytosine residues, yielding single-nucleotide resolution information about the methylation status of a segment of DNA. Various analyses can be performed on the altered sequence to retrieve this information (Long-Cheng and Rajvir., 2002); the sequence of the gene was pasted which is methylated, the job was submitted, and the results were gotten as original sequences in the first row and the bisulfate sequences in the next row and the CpG sites are marked as ++ and the unmethylated cytosine residue was converted as uracil, and the methylated cytosine remained as same (http://www.urogene. org/methprimer/index1.html).

Modification of methylation activity of Ten eleven Translocase enzyme2 (TET2)

Inhibition of TET2 was done, because it is responsible for the hyper methylation of BDNF (it transfers the methyl group to the cytosine residue) which causes the type 2 diabetes (T2DM). The inhibitor selection for this TET2 was based on the literature search and in that some of the inhibitor compounds are in the phase 1 and phase 2 trail. (Corbet et al., 2017)The molecular properties of these inhibitors were analyzed using various softwares. Inhibition was done with the help of docking tools (Swiss dock and autodock vina).This docking was first done using Swissdock and the top three compounds were selected as the input for swiss docking and autodocking .Compounds selected for inhibition of TET 2 were: analogs of fumerate hydratase (FH), isocitrate dehydrogenase (IDH), and succinate dehydrogenase (SDH)(Corbet et al., 2017).

Inhibition of TET 2 using swiss dock

The SwissDock web site is available online at http:// www.swissdock.ch. The structure of the target protein, as well as that of the ligand, can be automatically prepared for docking. . In addition, the cumbersome syntax of the docking engine is hidden behind a clean web interface providing reasonable alternative sets of parameters as well as sample input files. All calculations are performed on the server side, so that docking runs do not require any computational power from the user. The interpretation of docking results and their integration into existing research pipelines is greatly facilitated by the seamless visualization of docking predictions in the UCSF Chimera molecular viewer, which can be launched directly from the web browser(Yuriev et al., 2013).

Web interface Inputs. Only three steps are required to start a docking assay through the web interface of SwissDock: users must define a protein structure, one or several putative ligands and docking parameters .They are guided throughout this short and simple submission process by a comprehensive contextual help. several sample files are supplied to users and can be directly uploaded into the form simply by clicking on a link. The corresponding sample output files are also provided. Target selection. A target protein structure can be determined either by specifying its identifier from the Protein Data Bank or by uploading structure files. The first option allows users who are not familiar with 3D structure files to start a docking assay with only a PDB code. If many PDB records are present for the same target, those with a high resolution and a ligand alike to the one that will be docked should be measured first(Yuriev et al., 2013).

When numerous chains are exist in the PDB file, the user can stipulate the identifiers of those on which the docking

will be done. If the structure file has been produced by nuclear magnetic response experiment, it must be edited first to keep only one structural model (Yuriev et al., 2013).For visual investigations, UCSF Chimera can be started by a single click, and the predicted BMs are automatically loaded in its ViewDock plugin It comprises PDB files for the compound, as well as the predicted BMs in the DOCK format.

Interaction analysis with the use of Autodock Vina

The AutoDock Vina tool permits running ligand-receptor docking calculations with AutoDock Vina, by either a web service provided by the National Biomedical Computation Resource (NBCR) or a locally installed copy of the program(Trott et al., 2010).Calculations are for a single ligand; database screening is not enabled. Docking results are shown automatically in ViewDock.The receptor and ligand structures should be opened as separate models in Chimera. If the receptor arrangement contains MSE (selenomethionine) residues, imperfect side chains, or atoms with different locations, running Dock Prep beforehand to correct those issues is recommended. The AutoDock Vina tool runs AutoDock accessory scripts locally to (further) prepare the structures, such as to add hydrogens if they have not been added already with Chimera(Trott et al., 2010).

From protein data bank, PDB Files were taken. TET2 PDB id is 5DEU .Compounds selected for interaction of TET 2 were: analogs of fumerate hydratase (FH), isocitrate dehydrogenase (IDH), and succinate dehydrogenase (SDH).(Corbet et al., 2017).

Results And Discussion

DNA methylation Analysis

The CpG site was identified from the MethPrimer software. Results were observed. Original sequences in the first row and the bisulfate sequences in the next row and the CpG sites are marked as ++ and the unmethylated cytosine residue was converted as uracil, and the methylated cytosine remained as same; from that we can find the methylated and the unmethylated molecules {Figure 2(A),2(B)}.

Inhibition using Swiss Dock

The best ligand structures with their binding energy and the interaction of these inhibitors obtained from Swiss dock is shown in Table 1. The best 3 ligand structure and its interaction with the target protein are shown in Figures 3,4 and 5.

Inhibition using Autodock Vina

Autodock offers the full range of speed vs. accuracy options, from the high-throughput virtual screening (HTVS) mode for efficiently enriching million compound libraries, to the standard precision (SP) mode for reliably docking tens to hundreds of thousands of ligand with high accuracy,to the extra precision (XP) mode where further elimination of false positives is accomplished by more extensive sampling and advanced scoring, resulting in even higher enrichment. XP does more extensive sampling than SP. This SP and XP uses rigid docking only; only the ligands structure got moved and docked to protein in different conformations. The top three ligand molecules obtained from the auto-dock result was given as the input to the autodock package and the XP and SP docking was done; the docking score and the glide energy were noted. The best 2 ligand structure and its interaction with the target protein are shown in Figures 5and6.

Ramachandra plot

The structure of the enzyme TeT2 and the BDNF protein was analyzed by SAVS server. the results are shown in Figure 1(a) and1(b).

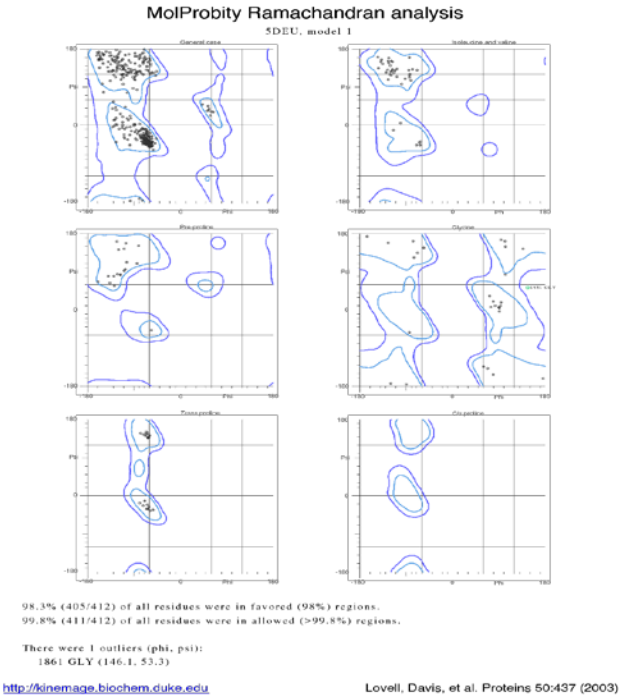

Figure 1(a) - Ramchandran plot of TeT2 enzyme

The structure of enzyme TeT2 with BDNF

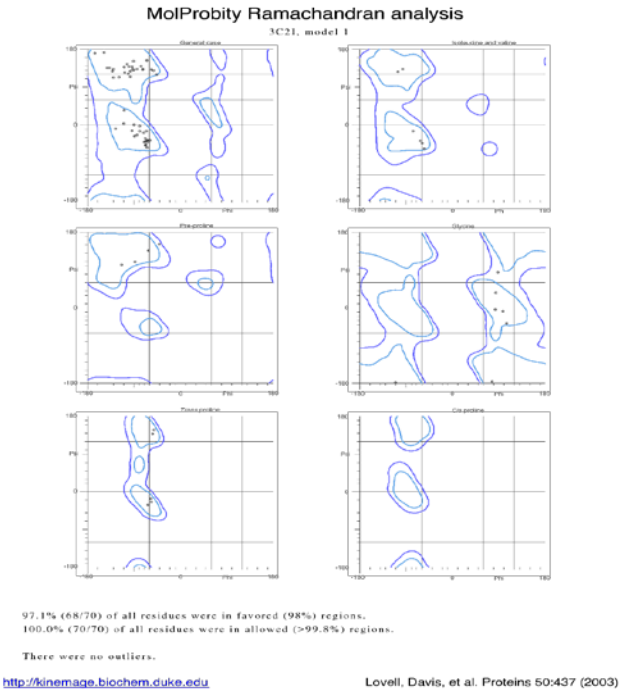

Figure 1(b)-Ramchandran plot of BDNF gene

DNA methylation analysis by methprimer

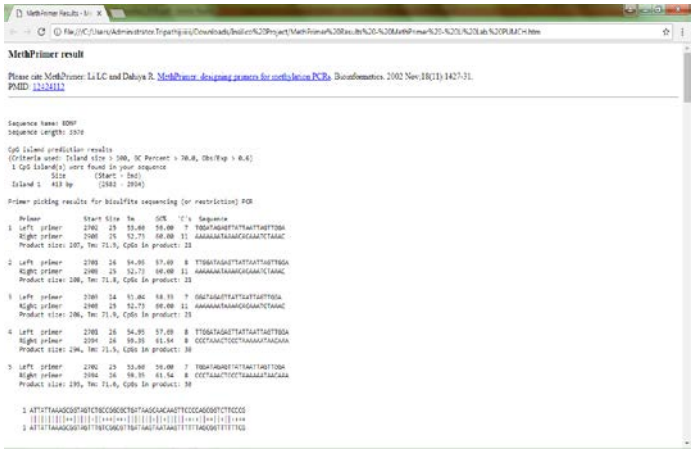

Fig.2. Visualization of primer selection results for MSP. CpG island prediction for primer selection was used as an input parameter. All other parameters were default values. (a) Graphic view showing primers and sequence features such as GC percent, CpG islands, and CpG site. (b) Text view showing sequence alignment and location of primers.

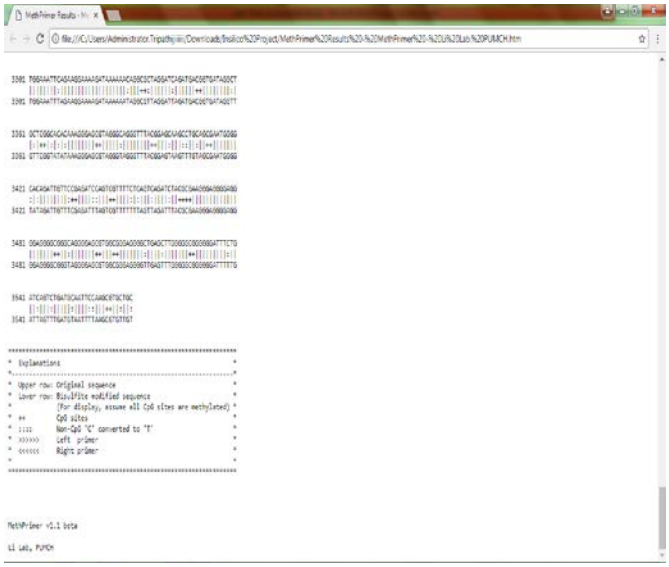

Fig.2(B) Methprimer result with Original sequence and Bisulfite sequence

Swiss Dock

1.Interaction with TET2(5DEU) and Fumerate Hydratase

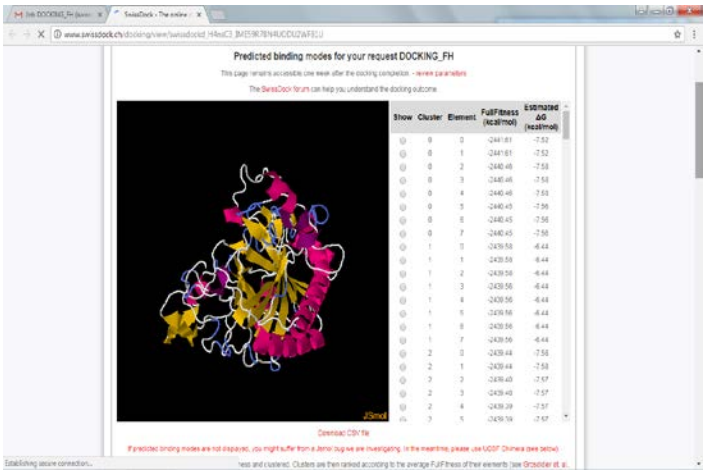

Fig.3 Visualization of docking TET2 with FH

2.Interaction with TET2(5DEU) and Isocitrate Dehydrogenase (IDH)

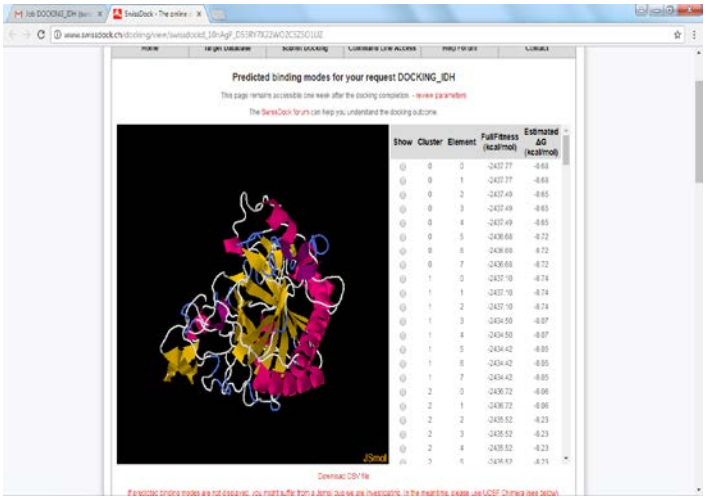

Fig.4 Visualization of docking TET2 with IDH

3.Interaction with TET2(5DEU) and Succinate Dehydrogenase (SDH)

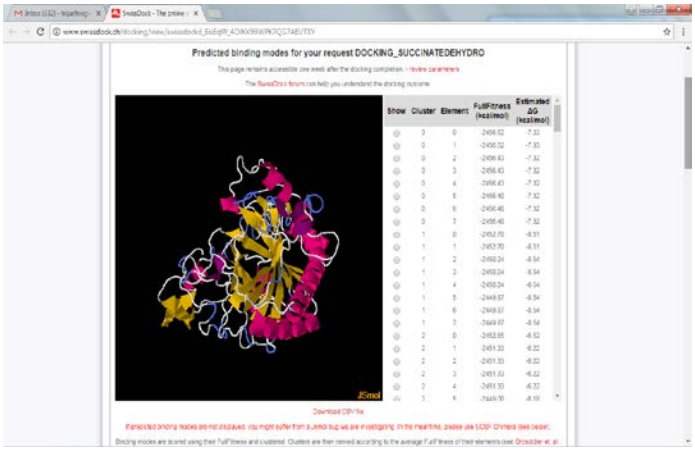

Fig.5Visualization of docking TET2 with SDH

Autodock vina

Docking with receptor(5deu) and ligand (FH)

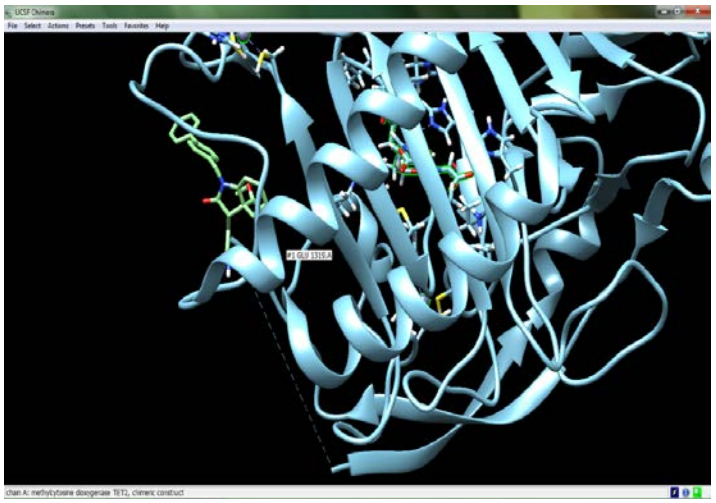

Fig.6 Visualization of docking TET2 with FH with the use of Autodock vina

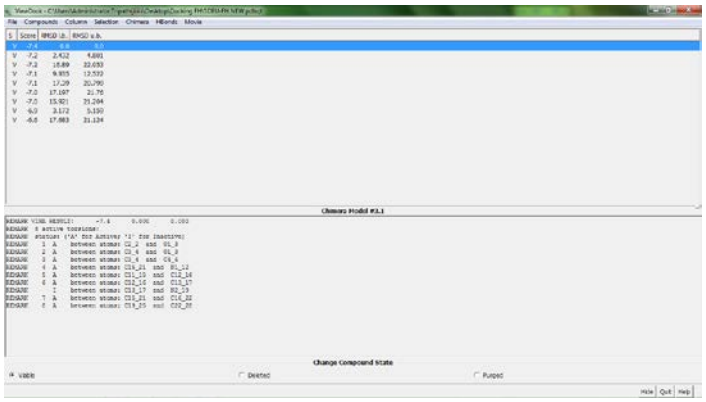

Fig.6(B) Docking result with Delta G in view dock

Docking with receptor(5deu) and ligand (IDH)

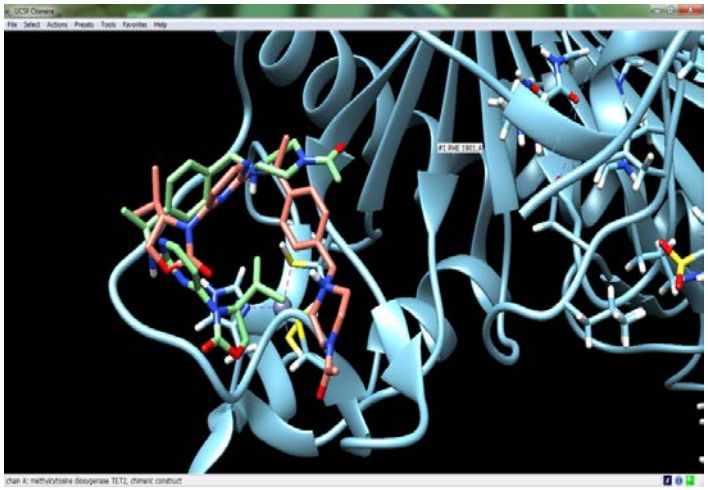

Fig.7 Visualization of docking TET2 with IDH with the use of Autodock vina

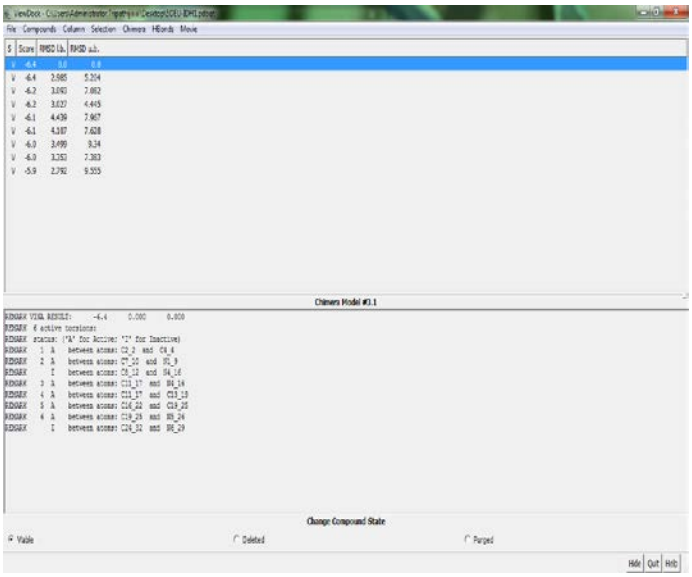

Fig.7(B) Docking result with Delta G in view dock

Interaction result with the use of Swiss dock

Protein and Ligand	G score
TET2 with FH	-7.52
TET2 with IDH	-6.68
TET2 with SDH	-6.3

Table(1) Shows the protein ligand with their G score with the use of swiss dock

Interaction result with the use of Autodock

Protein and Ligand	G score	H_2 Bond
TET2 with FH	-7.4	8
TET2 with IDH	-6.4	7

Table(2) Shows the protein ligand with their G score with the use of Auto dock vina

This above result shows the Ramachandran plot of TET2(5DEU) and the BDNF by the help of savs server, It provides a simple view of the conformation of a protein. Each residue will have two bonds that can rotate freely. These two angles define the conformation of that residue in a protein and are called the Ramachandran angles, ψ (psi) and φ (phi). It Checks the stereochemical quality of a protein structure by analyzing residue-by-residue geometry and overall structure geometry. Determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigned a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc) and comparing the results to good structures.

DNA Methylation of BDNF is analyzed by use of methprimer which shows the original sequence and bisulfite sequence , It design bisulfite-conversion-based Methylation PCR Primers. it can design primers for two kinds of bisulfite PCR: 1) Methylation-Specific PCR (MSP) and 2) Bisulfite-Sequencing PCR (BSP) or Bisulfite-Restriction PCR. MethPrimer also shows CpG islands in DNA sequences.MethPrimer takings results in both script and graphic view comprising results of primer picking and CpG island forecast it designing PCR primers for methylation mapping.These results were gotten as original sequences in the first row and the bisulfate sequences in the next row and the CpG sites are marked as ++ and the unmethylated cytosine residue was converted as uracil,and the methylated cytosine remained as same.

Swiss dock the docking tool with use of chimera shows the interaction,Molecular docking predict the structure of the intermolecular complex formed between three different molecules,Binding affinity quantifies the binding strength of a ligand to a protein

1.Interaction of TET2(5DEU) with Fumarate Hydrate(FH)

2. Interaction of TET2(5DEU) with Isocitrate dehydrogenase(IDH)

3. Interaction of TET2(5DEU) with Succinate dehydrogenase(SDH)

The difference ΔG in free energy of the bound state (all atomic arrangements where the protein is ligand-bound) and the unbound state (all atomic arrangements where the protein is not ligand-bound),A dissociation constant (Kd) which is (roughly) the ligand concentration at which half the protein molecules will have a ligand bound.

AutoDock Vina tool runs ligand-receptor docking calculations.Vina shows the interaction also with the hydrogen bond

1.Interaction of TET2(5DEU) with Fumarate Hydrate(FH)

2. Interaction of TET2(5DEU) with Isocitrate dehydrogenase(IDH)

Swiss dock shows the interaction&Molecular docking predict the structure of the intermolecular complex between the TET2 protein and FH,IDH,SDH.Same as the autodock vina shows the interaction between TET2 and FH,IDH,SDH.Interaction analyzed on the basis of DeltaG and also by the Hydrogen bond.On the basis of interaction we can say that TET 2 Protein best interact with IDH because it give the best interaction in swiss dock(-8.68).This ligand can also useful for therapeutic purpose or for the treatment of diabetes retinopathy.

Conclusion

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. 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. This result shows that isocitrate dehydrogenase (IDH) play an important role for in TeT2 protein and this shows best interaction (delta G= -8.68) with TET2 ,it might be helpful for controlling the TypeII diabetes retinopathy at the 5hmc region.

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