The Role of Epigenetic Changes in The Development of Diabetes Mellitus

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ABSTRAK

Diabetes melitus (DM) adalah masalah kesehatan yang paling penuh dengan tantangan dan dipercaya merupakan hasil antara interaksi gen dan paparan lingkungan. Terdapat hipotesis mekanisme epigenetik, menggunakan dasar molekular untuk menjelaskan mekanisme terjadinya DM. Karena paparan lingkungan termasuk status gizi dan keadaan hiperglikemia, risiko terjadinya DM telah dimulai sejak pre-konsepsi, yang berlangsung hingga dewasa, dan diwariskan antar-generasi. Terdapat 3 mekanisme utama epigenetik yang berperan pada DM. Mekanisme-mekanisme epigenetik juga memiliki peran dalam memori metabolik dimana komplikasi-komplikasi DM dapat tetap terjadi walaupun kadar gula darah dalam tubuh sudah normal. Pembatasan kalori dapat membantu menunda proses dan terjadinya penyakit-penyakit degeneratif termasuk DM dengan cara menstabilkan genom melalui mekanisme-mekanisme epigenetik.

Kata kunci: *diabetes, mekanisme epigenetik, memori metabolik.*

ABSTRACT

Diabetes mellitus (DM) is one of the most abundant diseases in the 21st century and believed as result of interaction between genes and environment exposure. There is a hypotesis of epigenetic mechanisms, using molecular basis to explain about the mechanism of DM. Because of the enviromental exposure including nutrition status and hyperglycemia state, the risk of DM has started since pre-conception, last until adulthood and will be inhireted trans-generational . Mainly, there are 3 epigenetic mechanisms that have role in DM. Epigenetic mechanisms are also have role in the metabolic memory that the DM complications may still developed although the blood glucose level is already normal. The restriction of calory intake may help delaying the development and onset of degerative diseases including DM by stabilizing genome through epigenetic mechanisms.

Keywords: *diabetes, epigenetic mechanisms, metabolic memory.*

INTRODUCTION

Diabetes mellitus (DM) is one of the abundant diseases in the 21st century. The approximated prevalence of DM among adults in the world was 366 million in 2011, and in 2030 this will become 552 million.[1] DM is a disease caused by metabolic dysregulation, marked by chronic high blood glucose level causing many complications that may reduce life expectancy and quality of life. The disease mechanism of DM is not well understood until now, but it is known that it is the result of interaction between

genes and environment exposure.[1,2]

There is a hypothesis regarding epigenetic mechanisms explaining the association between imbalanced nutrition and altered disease risk, using molecular basis to explain the link between perinatal nutrition intake and degenerative diseases, including DM.[2] The classical definition of epigenetic was first stated by Conrad Waddington in the 1950s, that mentioned the epigenetic trait as a stable phenotype changes that may be inherited, as the result from changes in chromosomes but with no alternation in DNA sequence.[3] Through epigenetics, the organism can alter its pattern of development to cope with environment stress, and this may pass intergenerationally as the accumulation of epigenetic modifications.[4]

INFLUENCES OF PRE-CONCEPTION, PRE-NATAL, AND POST-NATAL NUTRITIONAL EXPOSSURES AS RISK OF DIABETES MELLITUS TO THE OFFSPRINGS

Early life exposures bring a long period of life increased the risks for DM, and may cause persistent changes including metabolism. Intrauterine malnutrition influences organogenesis, which has bad effects on organ morphology and function.[5] The period of embriogenic development has been known as a critical window in establishing the epigenome.[6]

The exposures may cause trans-generationally inherited traits and epigenetic modifications. Trans-generational has a meaning that the the changes in phenotype must last for at least 3 generations after the first exposure.[7] The inheritance mechanism may happen through mother or father (P0). A pregnant woman (P0) can carry genetic and epigenetic information until F2 generation. Her fetus (F1) and its germ cell (F2) may be affected by enviromental expossures. The changes in F1 phenotype or epigenetic state to be considered trans-generational if it lasts until F3 generation. In males (P0), the expossure may change his germ cells (F1), but since the F2 germ cells are not expossed, every phenotype or epigenetic changes in F2 are defined as trans-generational.[7,8]

Paternal's nutrition intake can influence offspring's risk of diseases through sperm epigenome which is included heritable informations, like: DNA methylation. Paternal obesity has relationship with changes in sperm numbers, concentration, motility, morphology, and result in sperm DNA damage.[8] The paternal impaired fasting glucose and glucose intolerance, as manfifestation of pre-diabetes, will alter gene expression patterns in pancreatic islets, by downregulating several genes involved in glucose metaboism and insulin signaling pathway. It is hypothesized that nutrition supply as methyl donor may alter epigenetic reprogramming in sperm.[9]

Maternal pregnant obesity in combination with gestational DM may lead to newborn with hyperinsulinemia state and increased offspring's fat mass until the 6th week, and this is strongly suggest as an important influence of gestational DM to the offsprings. Maternal obesity during pregnancy will become a serious danger to offsprings in adulthood. It will start trascriptional programs that may increase the expression of inflammatory molecules and immune cells, which reflect the epigenetic proccess. Hyperinsulinemia state in response to maternal obesity is mediated by the Insulin Receptor Substrate-1 (IRS-1), which is regulated by epigenetic mechanism through short noncoding miRNA-126.[9,10]

Maternal nutritional deficiency or famine may also give influence to the offspring depending on the timing of their prenatal exposures. During the Dutch famine in the years 1944 until 1945, those who were concieved in the midst of famine had shown to have hypomethylation at CpG (Cytosine-phopshate-Guanine) islands inside important promoter site and it still can be observed until even 60 years later.[11] The intrauterine condition with poor nutrition will epigenetically program the offsprings to survive in a low nutritional postnatal environment, revealing 'catch-up growth' in postnatal period, and when they are exposed to nutrition which are high in energy, they will have obesity as a cause of insulin resistance state.[12]

EPIGENETIC MECHANISMS TO DEVELOP DIABETES MELLITUS

The epigenetic mechanisms are primarily occuring through three mechanisms. There are: DNA methylation, post-translational histone modification, and miRNA expression.[4]

DNA Methylation

The adult somatic cells consist of one haploid set of chromosome, which is inherited half from mother and the other half from father. Epigenetic patterns are at first deleted and reprogrammed two times to correct the imprinting sites with allele-specific methylation, to form tissue specific gene methylation pattern in postnatal periode. The 1st phase of deleting and reprogramming happened during gametogenesis. The DNA methylation is deleted and later reestablished by taking specific imprints in oocytes and in spermatozoa. The 2nd phase of epigenetic deleting and reprogramming happened during pre-implantation. During this proccess, the genome with possible exception of imprinted genes and some retrotransposons, is now demethylated. The DNA methylation is restored after implantation, and then forming cell descent specific patterns in rapid way to oversee its differentiation.[13] (**Figure 1**)

The DNA methylation is defined as the attachment a of methyl group to a cytosine (C) nucleotide at position 5 (5mC) and specifically happened when a cytosine's position is next to guanine and joined by phosphate in DNA, which is called by CpG dinucleotide. The regions of genome consists lots of CpG dinucleotide are called by CpG islands.[13] The frequent epigenetic

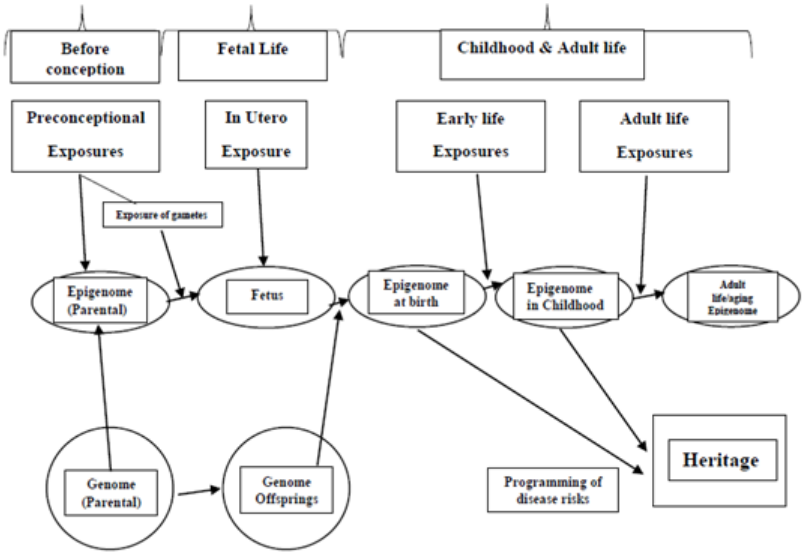

Figure 1. Exposure throughout steps of life and the epigenetic dysregulation. Environmental exposures may cause epigenetic changes, increasing the risk of diseases including DM. These changes are heritable to the offsprings.[13]

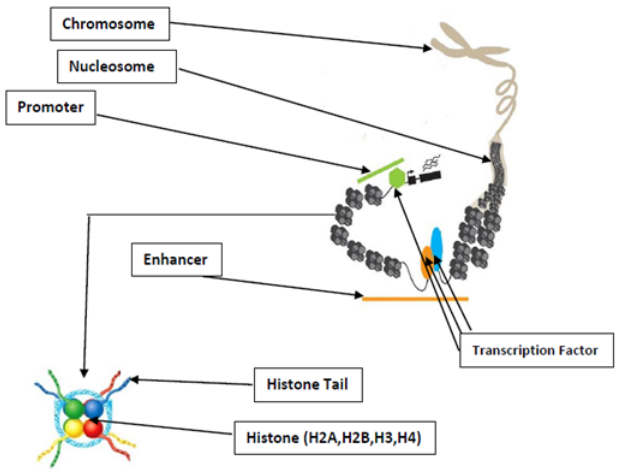

Figure 2. Landmark of regulatory regions and histone. The DNA methylation may take place at the CpG islands in the promoter region. An illustration of histones that consist of 4 types of histone with its tail.[5]

modification of DNA is in the demethylated CpG islands in promoter region. The promoter methylation during development and diseases proccess is related to gene trancriptional silencing.[5] (**Figure 2**)

The specific methylation of CpG dinucleotides in tissue is performed by DNA methyltransferase or DNMTs (1,3a,3b, and 3B) that has activity in de novo methylation (except for DNMT1 that has function to maintance the methylation of CpG), making the covalent binding of methyl group to form 5mC.[13,14] The demethylation proccess starts by the ten-eleven translocation methylcytosine dioxygenase1 (TET1), which is then oxydized 5mC to form 5-hydroxymethycytosine (5hmC),then into 5-formylcytosine (5fC), and then into 5-carboxylcytosin (5aC). The 5mC, 5hmC, 5fC, and 5aC may have passive demethylation to form C. The 5f and 5aC can be cut by thymine-DNA glycosylase (TDG), then followed by DNA repair (base excision repair or BER) to form C. As an alternative, the 5aC may react with decarboxylase to form C. The 5mC can be deaminated by activation-induced deaminase (AID)/apolipoprotein B mRNA editing complex (APOBEC) to become 5-hydroxymethyl Urasil (5hmU) then exicised by TDG or methyl-CpG-binding domain protein 4 (MBD4), and at the last followed by DNA repair to form C again.[14] (**Figure 3**)

The hyperglycemia state may induce global demethylation at all genomic loci including the promoter, intergenic, and intragenic sites. The most methylated CpG sites of diabetic islets show decrease in methylation.[15] This may be caused by the reduction of activity of DNMT1 during cell proliferation or a reduction in the activity of DNMT3a and 3b which responsible for methylation. There is also an increase in the action of Growth arrest and DNA-damage-inducible protein GADD45A (GADD45A) which may also cause demethylation in human pancreatic cells. These changes are risk factor for DM. Another explaination for hypomethylation in hyperglycemia state is due to the decrease in methyl donor.[16,17] The hyperglycemia state that may induce global demethylation is largely maintain in the metabolic memory state.[15] (**Table 1**)

Post-translational Histone Modifications

Histones (H1, H2A, H2B, H3, and H4) are globular protein which exist around DNA, packed to form chromatin. Post-translational histone conformational changes may happen

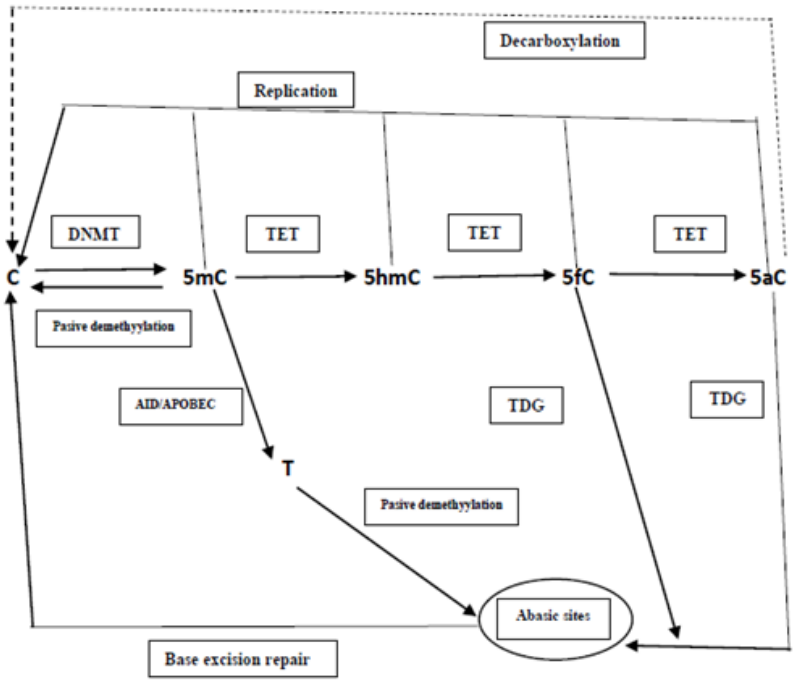

Figure 3. Model of DNA demethylation pathways initiated by TET. An illustration of the enzymatic changes among C, 5mC, 5hmC, 5fC, and 5aC.[14]

Table 1. Genes with expressions on metabolic process which are related to obesity and type 2 DM

Metabolic process	Genes	Epigenetic mechanisms
Adipogenesis	CEBPA	Histone aceylation and methylation
	PPARA	DNA methylation
Appetite regulation	LEP	DNA methylation
	MC4R	DNA methylation
	NPY	DNA methylation
	POMC	DNA methylation and histone Acetylation and methylation
Body weight homeostatis	FTO	DNA methylation
Glucose homeostasis	ADIPOQ	DNA methylation and histone acetylation
	GLUT4	DNA methylation and histone acetylation
	INS	DNA methylation and histone acetylation
Hypoxia	HIF1A	DNA methylation and histone Acetylation and methylation
Inflammation	IFNG	DNA methylation
	TNF	DNA methylation
Lipid storage	FASN	DNA methylation
Stress	NR3C1	Histone acetylation
Thermogenesis	UCP1	DNA methylation

These genes which have expressions on metabolic process that controlled by epigenetic mechanisms.

as the result of enzymes that modify lysine and arginine residues in amino terminus. The modifications are mainly acetylation and methylation. The acetylation may increase DNA accessability,meanwhile the methylation may result in either increased or decreased of DNA accessability, depend on the type of methylationing and histone.[14,18]

The histone methylations may happen at the lysine residue using SET- and non-SET domain histone lysine methyltransferase (HMTase) that regulates transcription and repair; and at arginine residue using protein arginine methyltransferase (PRMT) or coactivator-associated arginine methyltransferase1 (CARM1) that regulates transcription. The demethylation proccess may happen using lysine specific demethylase1 (LSD1). The acetylation may happen by using histone acetyl transferase (HAT) that regulates transcription, repair, replication, and condensation; and the deacetylation proccess using histone deacetylase (HDAC).[18-20] The active genes can be methylated at H3K4, H3K36, and H3K79, and the silenced or repressed genes are methylated at H3K9, H3K27, and H4K20.[21] The reversible acetylation of N-terminal lysine residues are at positions 9,14,18, and 23 of H3 and at 5,8,12, and 16 of H4. These reversible acetylation can mediate the decondensation of nucleosome structure, alters the link between histone and DNA, and also may give access for the binding of transcription factors.[18]

The miRNA Expression

The miRNA may work by blocking and altering the genes expression.[4] The target of miRNA is the gene promoter, through the recruitment of specific argonaute proteins to form epigenetic remodeling complexes. It can reduce gene expression by keeping histone deacetylation, histone methylation (H3K9 and H3K27), and DNA methylation.[22] The miRNA can bind to 3'untranslated region of target mRNA, reveling in degradation or inhibit protein translation.[4]

In the nucleus, RNA polymerase II is transforming miRNA to form long segmens of coding or noncoding RNA (pri-miRNA,consist of 70-100 nucleotides) which are covered up and polyadenylated, then captured and also extracted by a complex consists of RNase type III, drosha, and dsRNA binding protein, DiGeorge syndrome critical region gene 8 (DGCR8)' to form pre-miRNA, then to form a complex with exportin5 and RanGTP, and then it is translocated to cytoplasm. The dicer in cytoplasm can cleave the pre-miRNAs near hairpin loop to produce a short ~22 base pair long imperfect RNA duplexes. This short imperfect RNA dupleces are further added into mature RNA-induced silencer complex (mature RISC or MiRISC), and has a role as a template for capturing target mRNAs. The MiRISC can rend the target mRNAs through catalytic domain (RNA III domain) of argonaute protein (as the core component of RISC complex) and then degradating them.[23] The MiRISC may also prevent protein translation through several different mechanisms. These mechanisms are including the inhibition of trancription at level

of initiation and elongation, and the degradation of mRNA or the immature protein products of mRNA. The immature protein products of RNA are then segregated into P bodies to make inhibition of translational proccess and or deadenylation of mRNA. The final result is the destabilization of mRNA.[24]

The exposure of islet cells to palmitate can cause the increament of miR-34a and miR-146 which activate p53 and reduce vesicle-associated membrane protein 2. These proccess may lead to islet cells apoptosis and the reduction of insulin secretion. The exposure of proinflammatory cytokines to islet cells can activate miR-21,miR-34a, and miR-146, that may also lead to the reduction of insulin secretion and the increament of the apoptosis of the cells. The upregulated miR-29a and -29b in insulin target organs under hyperglycemic state may reduce insulin mediated glucose uptake.[25] (**Table 2**)

Table 2. The miRNA targets in type 2 DM

mRNA	Target	Function
miR-375	Myotrophin	Inhibition of insulin secretion
miR-9	OneCut2 transcription factor	Inhibition of glucose-stimulated insulin release
miR-192	E-box repressor	TGFβ-induced matrix protein collagen col a-1 and -2
miR-143	GLUT4, HSL, fatty acid-binding protein aP2, PPAR-μ2	Adipocyte differentiation

METABOLIC MEMORY

The long-(heritable) or short-acting (non heritable) environmental exposure may result in long- or short-term epigenetic changes.[26] The Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Intervention and Complications (EDIC) come with a conclusion that in DM patients who had improved glycemic control may still developed complications as result of prior poor glycemic control.[20] In 1990, Roy and colleagues had described the mechanism as metabolic memory, which is defined as epigenetic changes caused by hyperglycemia state-induced mitochondrial reactive oxygen species (ROS) production.[26]

Hyperglycemia state can reduced the activity of sirtuin1 (SIRT1) that can lead to the acetylation of p53, nuclear factor-kappa beta (NF-κβ) subunit p65, and histone 3 bound to p66shc promoter. The activation of p53 can lead to the increament of p66shc transcription. The reduction in the role of hyperglycemia state-induced GCN (an acetyltransferase) can cause the acetylation of H3 and the subsequent p66shc transcription through chromatin remodeling. The p53 protein that upregulates the p66shc can lead to the persistence of mitochondrial ROS production. The high ROS production may result in the endothelial dysfunction, vascular inflammation, and apoptosis proccess of cells.[27] Moreover, the high production of ROS may reduce the signalling proccess from insulin receptor substrate1 (IRS1) to phosphatidyl inositol 3 kinase (PI3K), and also activates c-jun N-terminal kinase that translocate pancreatic and duodenal homeobox-1 (PDX-1) from nucleus to cytoplasm and induces the degradation of mafA, resulting in the decreased of insulin gene expression,insulin production, and insulin secretion.[28,29]

Restriction of calory intake has already shown to give a positive correlation with disease prevention. This restriction may increase lifespan and delay cardiovascular disease, DM, and cancer, through chromatin remodeling which lead to increase in age and lifespan, and delaying the onset of degenerative diseases by stabilization of genome through epigenetic mechanisms.[9]

CONCLUSION

Diabetes mellitus is a metabolic disease characterized by high blood glucose levels, as the result of genes and environmental interaction. It is now accepted about the concept of epigenetic mechanisms by molecular basis to explain the development of DM. There are 3 main epigenetic mechanisms: DNA methylation, post-translational histone modification, and micro RNA (miRNA) expression. The epigenetic changes on the metabolic status are already there since pre-conception depending on the maternal and paternal epigenetic changes, and it will last

until adulthood, and the epigenetic changes will be inherited trans-generational. The epigenetic mechanisms are also involved in the metabolic memory, the condition where the complications of DM may still happen although the blood sugar level is already normal. Calories restriction may be benefit on delaying the development and onset of degenerative diseases including DM through epigenetic mechanisms to stabilize the genome.

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