Original Article

Basics of Epigenetics and Role of Epigenetics in Diabetic Complications

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Abstract

The term "Epigenetics" includes mechanisms by which genetic expression is altered without a change in the underlying DNA sequence. The changes caused by epigenetic mechanisms are inheritable and are one way in direction (irreversible) and also explains why there is differences in genetic expressions of monozygotic twins. The epigenetic mechanisms alter the genetic expressions through DNA methylation, posttranslational modifications (PTMs) of histone, and noncoding RNAs. DNA methylation and histone PTMs cause relaxation or condensation of chromatin units. The epigenetic actions of noncoding RNAs such as microRNAs, small nucleolar RNAs, small interfering RNAs, and long noncoding RNAs act by modifying transcription factors or by degrading target messenger RNAs and their translation factors. Various pathologies and environmental factors cause changes in the cellular epigenetic mechanisms and the epigenetic alterations occurring in diabetes mellitus (DM) are reviewed. DM causes hemodynamic changes and metabolic changes like hyperglycemia and dyslipidemia. These changes induce oxidative stress and activate intracellular signaling and kinases in the target cells. Epigenetic alterations cause chromatin remodeling and altered gene expression leading to inflammation, proliferation, atrophy, hypertrophy, etc.; thereby, diabetic complications such as neuropathy, nephropathy, vasculitis result in the corresponding target organ. When these epigenetic alterations persist for a longer period without intervention, the target cells attain "metabolic memory" meaning that these epigenetic mutations cannot be reversed even after attaining normal blood glucose levels. Thus, epigenetics, an insightful and efficient tool in genomic research, has started crawling into the research arena and needs to reach leaps and bounds for the better understanding of health and diseases.

Keywords: *Diabetes mellitus, DNA methylation, epigenetics, histone modification, long noncoding RNAs, microRNAs, noncoding RNAs, small interfering RNAs, small nucleolar RNAs*

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Introduction

James Watson and Francis Crick in 1953[1] discovered the basic structure of DNA, which revealed the greatest genetic secret behind life regulation and made a great breakthrough for understanding health and disease development. Since then, many of the diseases transmitting through generations were believed to be of genetic origin, but studies on monozygotic twins exhibiting varying genetic expressions (who were believed to be with the same genetic makeup) have changed the direction of the thought process. Historically, the new term "epigenetics" was coined then (Conrad Waddington[2]) to describe changes that could not be explained by genetic principles. With advancements in many of the molecular techniques, the genomes and genetic expressions are well understood, thereby defining epigenetics as changes that alter the genetic

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expression without change in the underlying DNA sequence. These epigenetic changes are transferrable to the next generation and are irreversible. In Greek, the term "epi" in epigenetics means "on the top of" or "in addition to" genetics.[3] The epigenetic mechanisms along with chromatin are responsible for lending cellular memory with cell fate plasticity and also for metabolic memory.[3]

Basics of Epigenetics

Structure of chromosomes

To know epigenetics, let us look into the basic arrangement of chromosome, which is a rod-shaped structure located in the nucleus, containing hereditary information. Chromosomes are formed by the condensation of chromatin and include deoxyribonucleic acid (DNA), histones, and other nuclear proteins.[1-5] [Figure 1].[1,4]

DNA, the basic human genetic material, is structurally a double-stranded helix composed of paired nucleotides [(adenine (A)-thymine (T) and guanine (G)-cytosine (C)], that are arranged on the inner staircase model and bonded by deoxyribose sugar and phosphate bond on the outer aspect[1] [Figure 1]. The coded information on these nucleotide pairs directs the synthesis of messenger RNA (mRNA) (transcription), and through this mRNA, coded information is passed off from the nucleus to the cytoplasm. In the cytoplasm, aminoacids are picked up from the ribosomes as per the instructions coded on these mRNA (translation) and proteins are synthesized. The proteins thus synthesized include enzymatic proteins and nonenzymatic proteins (e.g., structural proteins and hemoglobin) and they vary according to the secreting cell type, time of development, and location (e.g., ameloblasts secrete amelogenins)[1] [Figure 2].[6]

The double-helixed DNA strands are wrapped around the histone core. Eight core histones (two copies of each of H2A, H2B, H3, and H4) and the 146 basepair (bp) length of the wrapped DNA segment form a single nucleosome, which is the basic unit of chromatin. Two nearby nucleosomes are connected by linker DNA (generally 54 bp in length) and a single histone (H1). Thus, chromatin has a rigid structure and it can be condensed or relaxed by DNA methylation or posttranslational modifications (PTMs) in histone. The chromatin fiber so formed unites to form chromosomes[1] [Figure 1].

Epigenetic mechanisms

Epigenetics is really a very broad terminology, encompassing various mechanisms. With our present scientific probes, we have explored three important epigenetic mechanisms, namely (1) DNA methylation, (2) histone PTMs, (3) noncoding RNAs including microRNAs (miRNAs), small nucleolar RNAs (snoRNAs), long noncoding RNAs (lncRNAs), and short interfering RNA (siRNAs). Epigenetic mechanisms involving the nonhistone proteins can indirectly affect the gene regulation.

DNA methylation

DNA methylation[1-5] means the addition of a methyl group to the cytosine–phosphate–guanine (CpG) site

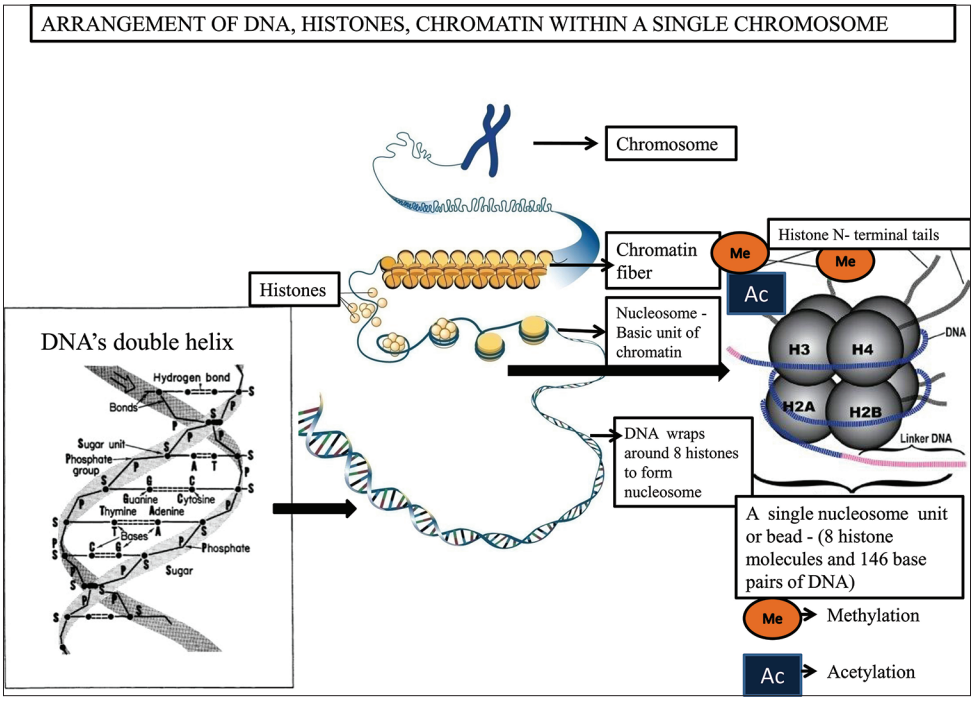

Figure 1: Arrangement of DNA, chromatin, histones within a single chromosome[1,4]

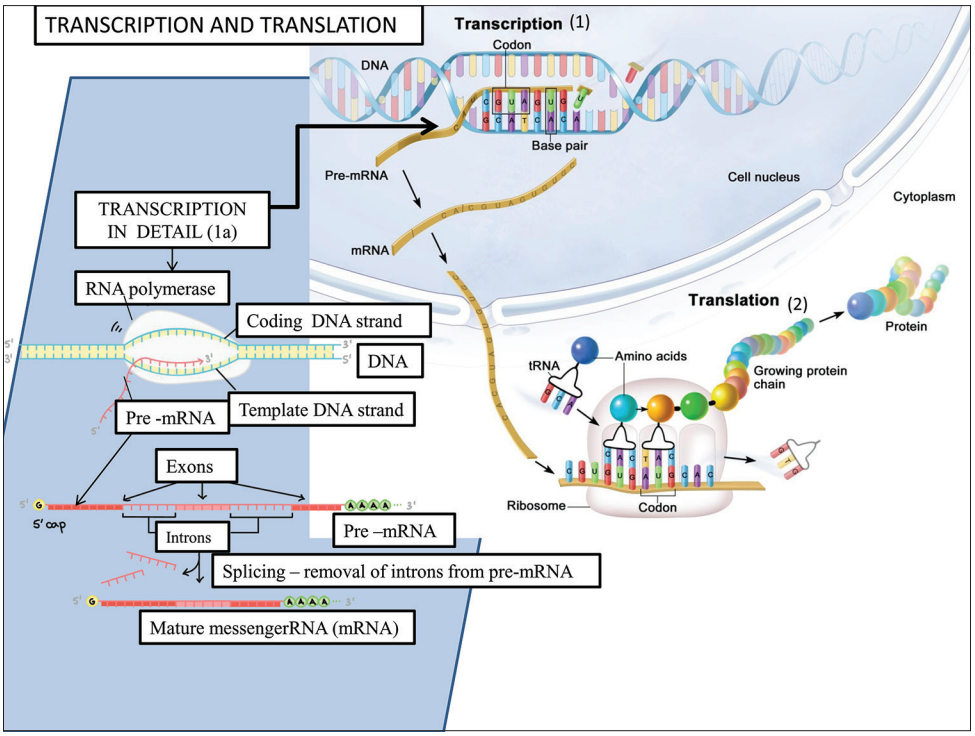

Figure 2: Transcription and translation[6]

of the DNA chain [Figure 3a]. The methyl donor is S-adenosyl methionine and the enzyme DNA methyltransferases (DNMTs) mediates the methylation process.

CpG sites (CpG islands) are located throughout the mammalian genome and they are usually nonmethylated, acting as gene promoters region. Once DNA methylation is accomplished, it serves two main purposes.

1. The methyl groups in the methylated CpG sites stick to the major grooves of DNA and block the binding of transcriptional factors to the DNA, thereby preventing transcription
2. The methylated sites also attract methyl binding proteins (methyl-CpG-binding domain proteins [MBDs]) located in chromosomes. MBDs proteins, in turn, assemble the enzyme histone deacetylases, which remove the acetyl group from histone. On deacetylation, the histone wraps the DNA more tightly, leading to chromatin compaction and gene silencing. It is the most characterized type of chromatin modification.

These DNA methylation patterns affect gene expression strongly, and these epigenetic patterns are conserved through cellular progeny when DNA replicates. Abnormal methylation states such as hypomethylation or hypermethylation can be initiated by environmental factors such as inflammation, hyperglycemia, hypoxia, diet, and smoking and can therefore lead to disease development. Hypermethylation of CpG islands causes gene silencing and hypomethylation causes activation of gene transcription.[3]

Histone modification[1-5]

PTM of histone protein is a powerful epigenetic mechanism because gene expression can be altered by chromatin condensation or relaxation. This condensation or relaxation state is achieved by acetylation or methylation of histone [Figure 3b].

Histone acetylation

Histone, on acetylation, helps in relaxation or opening up of the chromatin structure and facilitates transcription. The acetylated N-terminal ends of histone proteins protrude from the nucleosome core and recruit the basic transcription factors easily. On the other hand, deacetylation of histone removes the acetyl group and makes chromatin more tightened, inhibiting gene transcription.

Histone methylation

Histone methylation can cause either relaxation or condensation of chromatins, thereby causing activation or repression of transcription factors, respectively.

Noncoding RNAs[1-5]

Ribonucleic acids (RNAs) within mammalian cells are broadly classified as coding RNAs and noncoding RNAs (ncRNAs). Coding RNAs include mRNAs that are involved in coding of proteins and ncRNAs are a group of RNA molecules that do not code for a protein but perform other relevant functions. ncRNAs include

the following RNAs, namely (1) translational-related ncRNAs (e.g., transfer RNAs and ribosomal RNAs [rRNAs]), (2) small ncRNAs (length <200 bp) (e.g., miRNAs and short interfering RNAs [siRNAs]), and (3) lncRNAs (length >200 bp up to 100 kilobasepair [kbp]) [Figure 4]. Of these ncRNAs, the epigenetic roles of snoRNAs, miRNAs, siRNAs, and lncRNAs are well studied. These ncRNAs directly decrease the mRNA level and inhibit protein production by causing either degradation of target mRNAs or repressing their transcriptional factors.

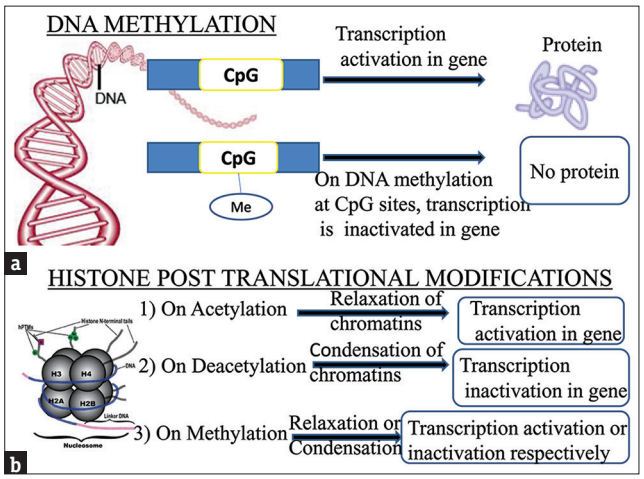

Figure 3: DNA methylation and posttranslational modifications in histone

Source of noncoding RNAs[1-5]

Gene is a part of DNA that contains the information to form a protein. Introns and exons are nucleotide sequences within a gene. When transcription is initiated through RNA polymerase, the portion of pre-mRNA, transcripted from from intron portion of gene, is eliminated subsequently (splicing). After the removal of the intron portion of pre-mRNA, the exon portion of pre-mRNA (transcripted from exons portion of DNA) is united to form mature mRNA and gets translated for protein formation [Figure 2]. The transcripts derived from the spliced introns give rise to almost all snoRNAs and majority of miRNAs. ncRNAs are also derived from transcripts of protein-coding exons.

MicroRNAs[1-5]

miRNAs are very small ncRNAs, made up of only 20–24 nucleotide, regulating transcriptional and posttranscriptional gene expressions. miRNAs are derived from the introns and exons of both protein-coding and noncoding transcripts and are synthesized by RNA polymerase II. After transcription, when mRNAs are formed, these miRNAs bind to the 3' untranslated region (3'UTR) of their target mRNAs, causing degradation of the target mRNA or suppress the expression of translation factors [Figure 4]. The vital role of miRNAs in various cellular differentiation process (e.g., odontoblastic differentiation) and diseases (e.g., oral cancer, oral immunology, and syndromes) is well studied.

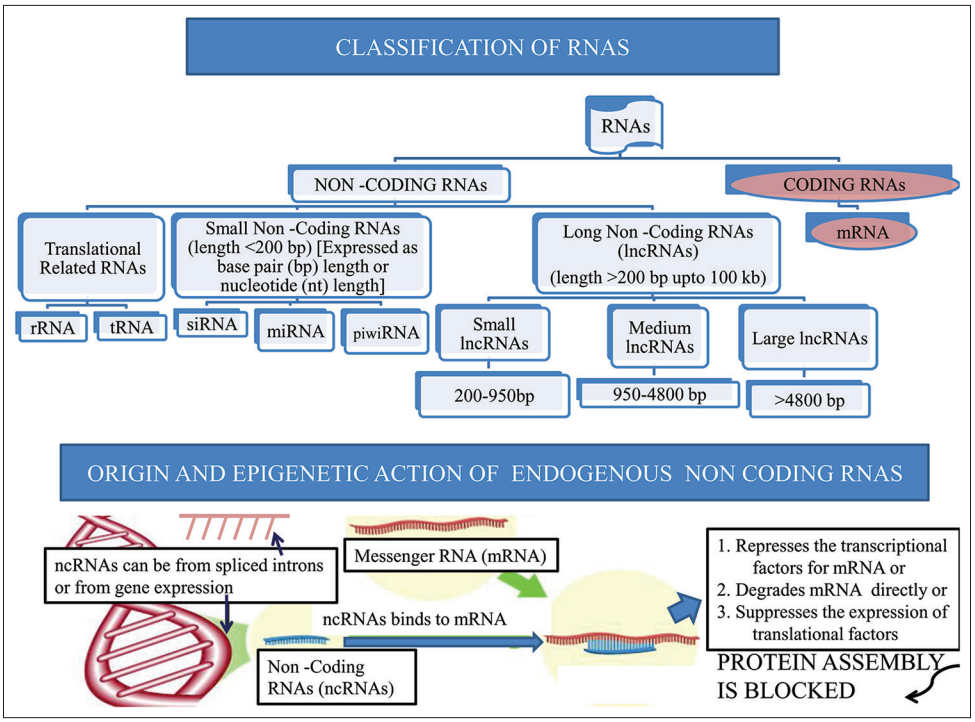

Figure 4: Noncoding RNAs– classification and mechanism of action

Small interfering RNAs[1-5]

Small interfering RNAs (siRNAs) are also known as short-interfering RNAs or silencing RNAs. They are very small, with length of about 20–27 nucleotides. Unlike miRNAs, which are single stranded and are produced within a cell (i.e., endogenous in origin), siRNAs are double stranded and exogenous in origin (not produced within the cell) and they enter the cell via vectors like viruses (active targeting) or through carrier complex (passive targeting). Through endocytosis, it is engulfed within a cell and gets released into the cytoplasm via lysosomal degradation. It is most notable in causing RNA interference through the formation of RNA induced silencing complex (RISC). RISC is a multiprotein complex (including proteins such as Dicer, Argonaute) that incorporates one strand of siRNA (or sometimes miRNA) and this single strand of siRNA acts as a template for recognizing complementary mRNA. Thus, RISC binds to the complementary sequence of mRNA, and one of the proteins in RISC called Argonaute activates RNAase and causes mRNA degradation[7] [Figure 5].[7]

Small nucleolar RNAs[1-5]

snoRNAs are about 60–300 nucleotides in length. Due to their small size and since they were initially identified in the nucleolus, during the biogenesis of ribosomes, they got the name snoRNAs. However, few of them are also identified in the Cajal bodies, which are nuclear organelle and are called as small Cajal body RNAs. They modify the target RNAs (mRNAs and rRNAs) by guiding site-specific modification of nucleotides through short regions of base pairing. They are of two major classes, (1) the box C/D snoRNAs which guide 2'-O-ribose-methylation and (2) the box H/ACA snoRNAs which guide pseudouridylation of target RNAs. Few snoRNAs are discovered without known target RNAs and are called orphan snoRNAs. They have a regulatory function and are involved in tissue-specific developmental regulation. Their aberrant expression is noted in syndromes.

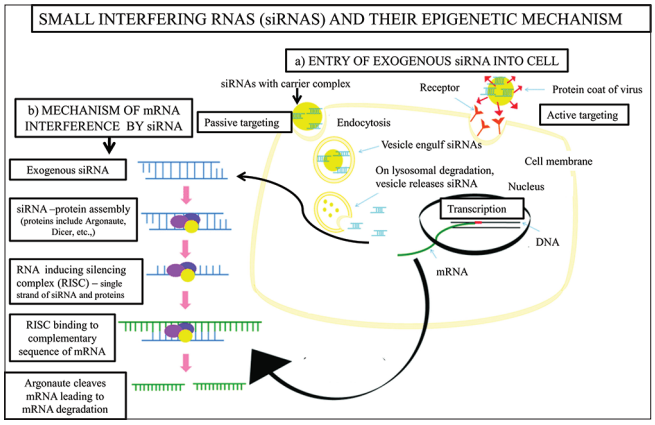

Figure 5: Mechanism of action of small interfering RNAs[7]

Long noncoding RNAs[5]

lncRNAs are >200 nucleotides in length and are classified as small (200–950 bp), medium (950–4800 bp), and large (>4800 bp) lncRNAs according to their length [Figure 4]. They are similar to mRNAs in length but are noncoding. They epigenetically act by affecting chromatin condensation, causing modulation of DNA methylation, and by modifying transcription factors. They are involved in numerous biological functions, including tumor suppression [e.g., MEG 3, GAS5, CCND1, long intergenic noncoding RNA-p21 (linc RNAs)], regulation of oncogenes (e.g., HOTAIR, MALAT 1, ANRIL, and SRA), and both tumor suppression and oncogenic (e.g., H19). They are also involved in transcription, differentiation, cell-cycle control, and immune response. Their dysfunctions are well proved to cause cancers and diabetic complications [lncRNA E330013P06[8] (also known as MIR143HG)].

Functions of Epigenetics

The epigenetic mechanisms play a vital role in genomic imprinting, organogenesis and development, cellular responses to environmental factors, inflammation, cancer, cell identity, stable inheritance of gene expression patterns in differentiated cells, X chromosome inactivation, stem cell plasticity, cellular memory, metabolic memory, and differential disease susceptibility between monozygotic twins.[2,5]

Role of Epigenetics in Diabetic Complications

The faithful propagation of epigenetic information is as important as the genetic information, because of which the precise regulation of biological process over multiple cell divisions is possible. Various environmental factors (diet, smoking, aging, inflammation and other stimuli) and stochastic patterns are known to induce epigenetic defects that play a major role in occurrence of complex diseases, including diabetes mellitus (DM).[5,8]

DM is worldwide increasing in occurrence and its prevalence rate is around 450 million people at present. Due to the sedentary lifestyle, lack of physical work, over nutrition, unhealthy diet pattern, etc., the incidence rate is expected to double in 2030. It is high time that efficient preventive measures should be taken and understanding the molecular mechanisms is very essential for early diagnosis, prevention, and treatment of this silent sweet killer.

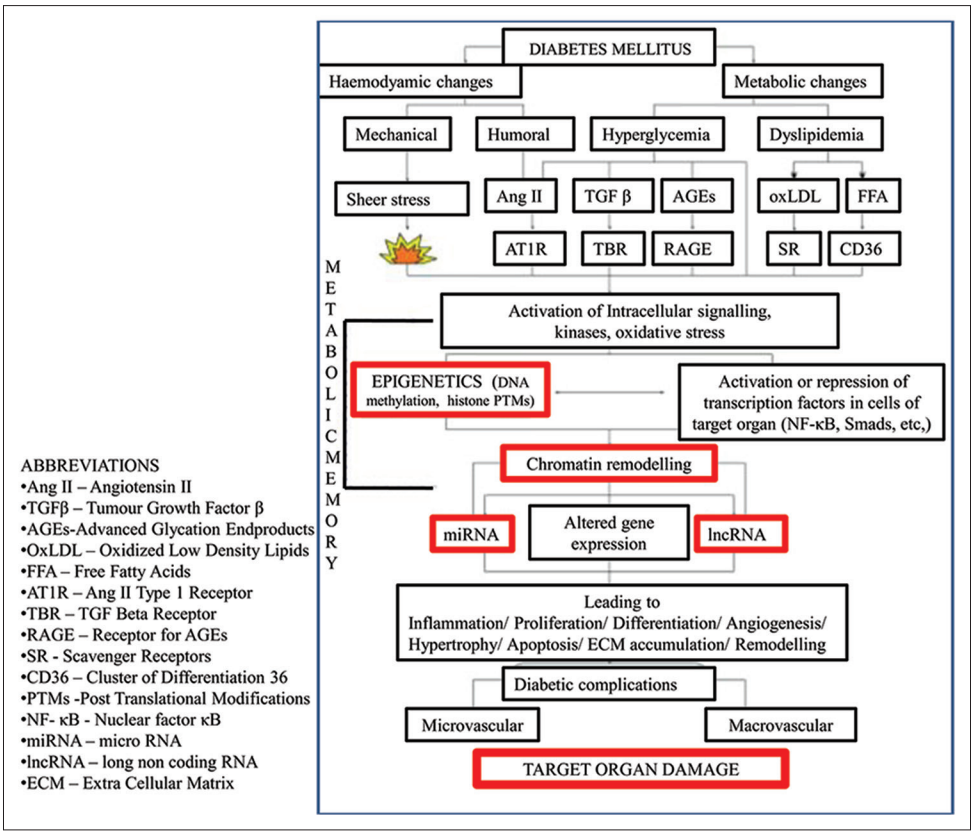

Figure 6: Role of epigenetics in pathophysiology of diabetic complications[8]

Epigenetics, an insightful and efficient tool in genomic research, has brought many of the molecular level changes occurring in the target cells suffering from DM into the limelight. The epigenetic defects are well established to be involved in the etiopathogenesis of DM as well as in the pathologies of diabetic complications.[5,8] [Figure 6].[8]

Modulation of growth factors, oxidative stress, and chronic inflammation are the basic cellular mechanisms by which diabetes affects almost all the organs of the body. Oxidative stress and deregulation of growth factors are caused in cells of target organ due to hemodynamic changes and metabolic changes induced by DM, thereby leading to epigenetic mutations. Henceforth, the cells of target organs like endothelial cells and vascular smooth muscles and retinal, neural, renal, and cardiac cells undergo vascular complications, retinopathy, neuropathy, nephropathy, and cardiac diseases, respectively. Although these complications are commonly depicted, the specific genes regulated/deregulated, changes in genetic expressions and pathologies vary according to the individual target organs.[5] Interestingly, cross talk occurs between these epigenetic layers (eg., ncRNAs and chromatin) in diabetes patients.[9] Furthermore, there exists a cross talk between epigenetic and genetic regulatory mechanisms, which can amplify or decrease the expression of pathogenic genes causing diabetic complications.

Chronic inflammation is the potential vascular complications of diabetes. In vascular cells (endothelial cells and vascular smooth muscle cells) and inflammatory cells (monocytes, macrophages, and lymphocytes), high glucose activates the nuclear factor-κB (NF-κB) pathway in the inflammatory genes.[10] Several epigenetic mechanisms including histone PTMs are explored in the activation of inflammatory genes and are tabulated in Table 1.[10-14]

When these altered epigenetic mechanisms persist for a longer time because of continuous high blood glucose level, it lends "metabolic memory", meaning that vascular and target organ damage at molecular level continue, even if the diabetic person attains normal blood glucose level later.[15] Thus, when diabetes patients are interrupted earlier with intensive treatment, many of these potential complications can be reverted without attaining metabolic memory and the benefits of this early intensive intervention last for a longer period of time even after cessation of treatment (legacy effect).[16] Metabolic memory is well demonstrated through the cell culture studies of anti oxidant and inflammatory genes in the endothelial cells, vascular smooth muscle cells, fibroblasts, etc., and also in animal models of diabetic neuropathy and diabetic nephropathy. [14,17-24]. Epigenetic mutations are proved to be related to the establishment

Table 1: Epigenetic mutations causing inflammation due to diabetes mellitus

	Target cells for epigenetic mutations	Epigenetic mutations	Functions	Clinical applications/relevance
Inflammation	Monocytes	Increases levels of H3Kac and H4Kac at inflammatory gene promoters.[8]	Enhance chromatin relaxation and inflammatory gene expression	High glucose-mediated activation of Nuclear Factor Kappa B (NF-κB) is a major mechanism of inflammatory gene expression in vascular cells and monocytes
		Differential enrichment of H3K4me2 (active) and H3K9me2 (repressive) marks[9]	Indicates cross talk between genetic and epigenetic factors	Protein SET is a protein in humans that is encoded by SET gene (nuclear proto oncogene). SET7 is required for the maximal activation of a subset of NF-κB-inducible inflammatory genes in monocytes
		Variations in H3K9/14ac, H3K4me2 and H3K9me2[8,9]		Histone PTMs and SET7 are the key epigenetic regulators of gene expression in many cell types
		Variations in monocyte H3K9ac at two HLA genes with single nucleotide polymorphisms, closely linked to type 1 diabetes mellitus[10]		SET7 inhibitors could be evaluated for the treatment of diabetic complications.
	Vascular endothelial cells	Promoting SET7 recruitment and H3K4me1 enrichment[11]	Increase the expression of the NF-κB active subunit (p65) and inflammatory genes	Down regulation of gene expression related to IL-6 and activator protein1 (AP-1) signaling pathways in endothelial cells treated with two arginine methyltransferase inhibitors, had inhibitory effects on SET7
	Vascular smooth muscle cells	Decrease in the repressive mark H3K9me3 and corresponding HMT SUV39H1 at their promoters[7]		

of metabolic memory,[14,17-24] and earlier good glycemic control can prevent various diabetic complications.

Since the epigenetic mutations are inheritable, it also plays an important role in transmission of these diabetic changes to the successive generations. The most attentive thing is that these epigenetic alterations are impregnated even when the child is in the foetal stage and continue to modify the child's metabolisms as he grows up, causing clinical type 2 DM in the later stage of life (fetal origin of adult diseases hypothesis).[25] Gestational diabetes also affects the organogenesis in the fetal stage because of maternal hyperglycemia and is rightly described as one of the potential "teratogens" for the fetus. Furthermore, type 1 DM has preclinical epigenetic mutations in the fetal stage itself. Epigenetic mechanisms are also responsible for delayed wound healing observed in diabetic patients.[26]

DM is a multifactorial disease and can be transmitted through generations. As a preventive measure, changes in the lifestyle like exercises[27] and healthy diet can alter these epigenetic mutations, reinforcing the role of physical measures in preventing diabetes and its complications. High blood glucose activates inflammation in almost all the organs and the epigenetic mutations occurring in these patients can be evaluated by analyzing inflammatory cells like monocytes, lymphocytes in blood.[10-14] In addition, most importantly, miRNAs are stable in biological fluids such as urine and serum[11] and can be noninvasively accessed for evaluation of newer biological markers. New biomarkers and drug targets for early intervention of diabetes (eg, anti-miRNAs)[11] are in the top priority of epigenomic research. Furthermore, a thorough research in this arena can lead to individualization of treatment to the diabetes patients, for example, identification of epigenetic mutation and its deactivation (e.g., demethylation of hypermethylated gene) using epigenetic tool is possible which, in turn, can intervene the progression of diabetes. Thus, early intervention and prevention of diabetic complications is possible with a deeper knowledge of this promising tool.

Conclusions

Basic epigenetic mechanisms act by DNA methylation, histone PTMs and ncRNAs (miRNAs, lncRNAs, siRNAs, snoRNAs). They cause changes in genetic expression by causing condensation or relaxation of histone or modifying transcriptional factors, degradation of mRNAs, or altering translational factors. Thus, protein formation is intervened and genetic expression is altered. The basic epigenetic mechanisms are deregulated by DM in various target organs and metabolic memory is established through these epigenetic mutations if high blood glucose level is left uninterrupted. DM lays its epigenetic signature in all the target organs through chronic inflammation, oxidative stress, and alteration of growth factors. Epigenetic changes in DM play a significant role in delayed wound healing and transmission of disease through generations. The early identification and deactivation of mutated

epigenetic mechanisms is possible through advanced epigenomic tools, which can be the most efficient management measure for diabetes and its complications in the near future.

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Conflicts of interest

There are no conflicts of interest.

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