Diabetes—Role of epigenetics, genetics, and physiological factors

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Abstract: Cells of organ systems are endowed with a relatively similar genome while epigenome niches keep varying chronologically and defined explicitly in the respective tissues. The genome of an individual is always influenced by parental, embryonic, tissue-specific, and environmental epigenomes and the same must have been the possible reason for invariable inquiries relating to familial, environmental and life style patterns in the preliminary investigations of diabetic complications. Unprecedented methylation of lysine residues of histones and cytosines of CpG islands of promoter DNA impede the transcription of genes and homocysteine is the metabolic key player of methyl groups. Gck and COX7A1 are the 2 examples in the present review to elucidate the epigenetic influence on the onset of diabetes. miRNAs are additional promising cellular components influencing both at transcriptional and translational levels and promoting either in favour or against (i.e., feed back) TFs, signaling factors and proteins through their ploiptropic effects and thus are reported to regulate cellular physiology. miR-124a and miR-9 are primarily endemic to nervous tissue and they are now being exploited in islets for their function in executing exocytosis of insulin, which of course is one of the fundamental canons of diabetes. miR-375 persuades beta cells for glucose-induced insulin gene expression. The current approach to evaluate the constellation of genes and their products involved in diabetes in huge number of samples through GWA studies may unravel intricacies involved in the management of diabetes and its associated consequences.

Key words: epigenetics; miRNAs; genetics; genome wide association

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1 INTRODUCTION

The concept of epigenetics elucidated by Waddington\cite{1} reveals that genome of an individual is programmed chronologically and influenced by ontogenic changes. Late-onset of disease such as diabetes is widely prevalent among the members of human population. Several reviews\cite{2-4} and experimental evidences\cite{5-9} have thrown light on genetic, metabolic, and molecular aspects of glucose homeostasis and suggested a variety of drugs, such as, sulfonylurea, meglitinides, biguanids, thiazolidinediones, insulin, etc., to combat against glucose impairment and to minimise the consequential complications of diabetes. In the present review the significance of a few recently contemplated topics such as epigenetics, miRNAs, and GWA studies has been highlighted to revisit the subject of diabetes in relation to current research pursuits.

2 EPGENETICS

The World of Biology is revolting around genes and issues influencing their functions. Of late, one of the interesting phenomenons that have come into vogue is that, among eukaryotes single gene alone cannot manifest its function. Constitutively and conditionally, the function of a chosen gene is being dictated by its immediate environment. The genome of an individual is functional since its inception namely zygote stage which is being characterized by bestowing with a biologically dynamic package of gene transcripts\cite{10-13}. Despite the presence of a set of all genes even at the early stage of development, only a few selected genes are made to transcribe to meet the requirements during ontogenic development. In the early 20th century several embryologists were in fact puzzled to unfold the intriguing mechanism that blastomeres adopted during initial developmental phases of an organism. Intuitively, in this context, Waddington\cite{1} elaborated the term “epigenetics” as the casual interaction between genes and their products which bring the phenotype into being, while explaining the phases of chick embryogenesis. Currently, the scope of epigenetics is extended to ageing, a variety of pathogenesis and even to the point of therapy\cite{4,14-17}. Thus, there are intrinsic epigenetic factors in the genome of an individual. They are transitory during development and stable in post-mitotic stages and furthermore, they are being influenced by environmental cues, which constitute extrinsic epigenetic factors. Albeit the genome relatively remains same in an individual, however the genome keeps varying during ontogenic development of an individual and eventually executes a stable influence in highly differentiated cells. In addition, the transient epigenetic factors during developmental processes of an organism invest a bunch of behavioural and adaptive features for the organism which pave the way for its interaction with the environment. Unforeseen alterations in epigenetic factors in post-mitotic cells not only influence the gene rendering toward its malfunction leading to a variety of cellular pathogenesis such as cancer, but also prepare the ground for late-onsets of diseases. Thus, the impaired glucose tolerance in adults must have been one of the consequences of epigenetic influences.

The developmental cascade of epigenetic factors invariably begins in the phase of gastrulation with the release of signaling factors, viz., FGF, BMP, and Wnt\cite{18-20} in embryos endowed with regulative type of development. These factors are crucial for the onset of epigenetic niches, namely prospective organ forming areas. However, the typical morphogen paradigm shown in the axes development in Drosophila is one of the wonderful models of epigenetic niches\cite{21}, of course, represented in embryos with determinate developmental patterns. These epigenetic niches possibly establish lineages for organs and as the development proceeds lineages are getting restricted to mould physiological barricades and architecture of an organism. Thus, the genome of any post-mitotic cell (even the islets of Langerhans) is always under the
influence of 4 possible epigenomes: (1) parental epigenome that it gets inherited and sustained through (2) embryonic epigenome during developmental processes, (3) tissue-specific epigenome mend by prospective organ forming cells through assembling a molecular matrix for their cellular differentiation, and (4) environmental epigenome which influence the gene function (Fig. 1). The unprecedented induction of aberrant alterations in any one of the above epigenetic influences in the targeted organ system, viz., islets or hepatocytes/myofibrils leads to the lowering of insulin secretion or building resistance to insulin respectively as reviewed in the following sections.

![Embryonic epigenome](image)

Fig. 1 Cellular genome is influenced by several factors in a manner keeping pace with time and space.

2.2 COX7A1

Another interesting observation wherein DNA methylation of one of the enzymes involved in oxidative phosphorylation (OXPHOS) in muscles is reported by Szendroedi, et al., and Rönn, et al. Liver and skeletal muscles are universally 2 vulnerable hotspots for Type 2 diabetes. The previous example cited above highlights the function of hepatocytes in relation to age. In myofibrils of skeletal muscles, the chain of reactions in OXPHOS takes place predominantly in mitochondria, wherein electrons are carried from NADH and FADH2. The generated proton gradient thus is being used for the synthesis of energy molecule, viz. ATP from ADP. Rönn, et al. using biopsied human skeletal muscle analysed the expression of COX7A1 mRNA through RT-PCR and reported that mRNA expression in skeletal muscle declined with age. Further, they reported that DNA methylation in the COX7A1 promoter region increased in elder individuals compared with young and found 22 methylation sites in the promoter CpG island. An upsurge in DNA methylation in another OXPHOS gene, NDUFB6 is shown in aged individuals with a concomitant decrease in gene expression. Thus, age-related changes in epigenetic niches are the cause for late-onsets of diseases (Tab. 1) and incidentally Type 2 diabetes is one among them.
2.3 Chromatin and methylation dynamics

The double helical DNA is tightly wound around histone octamers as units of nucleosomes constituting building blocks of chromatin in eukaryotes (Fig. 2). These histone octamers covalently bind to DNA and make genes stable temporally and thus distribute them on the chromatin of the respective chromosomes in a species-specific manner. In a string of this chromatin, intermittently nucleosomes are either in compact or relaxed state due to the influence of dynamic chromatin modifications, which makes a portion of DNA accessible for transcriptional factors (Fig. 3). The post-translational changes in histones which are differentially being dictated during ontogenic development of an individual, determine the selection of genes for transcription. A few genes are developmentally precluded from transcription and the same is facilitated by chromatin modification into a transient state called heterochromatin, which maintains a condensed conformation for the chosen period of time and the chosen region of chromatin. Whereas, euchromatin is an explicit portion of the chromatin wherein nucleosomes are kept quite apart and thus DNA double helix connecting nucleosomes is relaxed (Fig. 2) and facilitates promoters to solicit transcriptional factors which in turn pilot the local genetic machinery into an active state of transcription. This mechanism is the crux in chromatin biology, around which the modern concept of epigenetics centers.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Cellular components</th>
<th>Promoter methylation induced insufficiency in enzymes</th>
<th>Impairment</th>
<th>Observable cellular symptoms</th>
<th>Phenotypic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocytes</td>
<td>Cytosol</td>
<td>Gck</td>
<td>Anabolic utilization of glucose</td>
<td>Reduction in glycogen Synthesis</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>Skeletal Myofibrils</td>
<td>Mitochondria</td>
<td>COX7A1</td>
<td>Glucose combustion</td>
<td>Reduction in ATP derived energy</td>
<td>Insulin resistance</td>
</tr>
</tbody>
</table>

Fig. 2 Condensed chromatin impediment for transcription (A) and relaxed chromatin soliciting transcription (B). Ac: Acetylation; DA: Deacetylation; DM: Demethylation; M: Methylation.

Fig. 3 Pictorial representation of epigenetic mechanisms influencing chromatin structure. B6 and B12: components of B-complex vitamin; HAT: Histone acetylation; HDAC: Histone deacetylation; HDEM: Histone demethylation; HME: Histone methylation; HCY: Homocysteine; M: Methionine; SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine.

DNA is the repertoire for biological information in an individual and allows its functional units to elicit in a chronological sequence. The information processing and the consequent manifestation of a phenotype are all determined through the state of
Chromatin and its modifications. Chromatin constitutes histone (H) proteins, non-histone proteins, and DNA in almost equal proportion. Histones of chromatin are of 4 types: H1, H2 (A, B), H3, and H4. H1 is the linker histone and does not form a part of an octomer and ubiquitously histone octomer comprises of a pair of each of the remaining histones. The positively charged lysine terminals of histones covalently bond with negatively charged phosphodiester bonds of DNA and keep nucleosomes in a compact state called heterochromatin. Of the 4 histone proteins forming an octomer, post-translational changes occurring in H3 play a prominent role. The putative sites for these changes to occur are: H2AK19, H3K4, H3K9, H3S10, H3K14, H3K27, H3K36, H3K56, H4K8, H4K16, H4K20, etc. Out of several post-translational changes, histones undergo either ubiquitination of H2AK19 which is essential for Polymerase II recruitment, trimethylation of H3K4 for transcriptional initiation or deacetylation and methylation of remaining sites to repress promoter sequences and on the contrary, phosphorylation and acetylation to favour the transcriptional activity. Those events which favour gene activity make the covalent bond free between histones (lysines/arginines) and phosphodiester bonds of DNA and thus respective promoter sequence is made accessible to transcriptional factors. On the other hand, histone methylation does not alter the charge of lysine and hence, the covalent bond between DNA and histone remains uninterrupted and retains a compact state, viz., heterochromatin. The histone code hypothesis\(^{27-28}\) elaborately elucidated the role of chromatin modification in the transcriptional expression of genes. It has been shown that promoter specific hypermethylation is associated with conditions such as aging, cancer, atherosclerosis, and diabetes\(^{10,26,29}\).

The source for methyl groups is primarily nutrition. In addition, vitamins such as B6 and B12 also contribute in the intricate network of transient stages of methyl group. Methyl groups in an organism keep mount up over the span of years and find themselves involved in the metabolic network and get shunted between homocysteine (HCY) and methionine. In the process, they take a detour and couple to form a transient compound, i.e., S-adenosylmethionine (SAM) which delivers methyl groups either to cytosine of DNA (mostly in CpG islands) or lysines of histones and get transformed into S-adenosylhomocysteine (SAH), thus keeping itself free to get enriched with methyl groups (Fig. 3). The key metabolite is homocysteine, which contributes as a major depot for piled up methyl groups. The level of HCY in circulating fluids has now become one of the clinical diagnostic parameters to evaluate the extent of histone/DNA methylation\(^{30}\). Therefore, nutritional epigenome determines the health of an organism.

3 MICRO RNA

Diabetic pathogenesis has been recently reported to be unequivocally intertwined with micro RNAs\(^{31}\). Micro RNAs (miRNA) are novel class of non-coding RNAs with 21–23 nucleotide-long that are now believed to regulate gene expression post-transcriptionally by either cleavage recruiting dicer enzyme or translation repression of target mRNAs. Approximately 500 miRNAs of humans are annotated and indexed in the micro RNA registry (http://microrna.sanger.ac.uk). miRNAs as key factors are associated with cellular signaling factors in the pathogenesis of diabetes, complications, and cancer\(^{32}\). The regulation of insulin production, exocytosis, metabolic function, gene expression, and beta cell development are found to be mediated by miRNAs. Their roles in glucose induced insulin secretion (Fig. 4), ontogenic development of pancreas, islets differentiation, and the regulation of transcriptional factors\(^{33}\) have been reported. miR-375, miR-124a, miR-29a, b, and miR-9 have been shown to be associated with pancreas, expression of several components of the exocytic machini-
nery and in turn glucose homeostasis\textsuperscript{[34]}. Each of these miRNAs has pleiotropic effects influencing a cascade of cellular signaling and ultimately mediating beta cell function. Mice with homozygous deletion of miR-375 appear to have hyperglycemic effect because of decreased total pancreatic beta-cell mass and insulin levels\textsuperscript{[33]}. miR-375 influences these effects through myotrophin and NF-kappaB. These 2 transcription factors (TFs) are involved in glucose mediated synthesis of insulin from beta cells of pancreas\textsuperscript{[5]}. miR-124 is abundant in neuronal cells and pancreatic islets similar to miR-9 in distribution and get involved in the beta-cell development for hormone production. miR-124 and miR-9 involve in the regulation of components modulating exocytotic process i.e., secretory function of islets (Fig. 5). These exocytotic components include both neuronal origin and endocrine secretory cells’ origin. The components of neuronal origin are also targets for REST transcription factor assembly\textsuperscript{[35]} which promotes the increase in the release of insulin secretion even at low glucose stimulation. Four exocytotic components including SNAP 25, Rab 3 a, Synapsin-1 A, and Noc2 improve cellular secretory properties\textsuperscript{[31]} and further they are up-regulated by miR-124 a. On the contrary, granulin, another inverse modulator of exocytotic process is under the control of miR-9 through onecut-2 transcription factor. Overexpression of miR-9 causes a decrease in glucose induced insulin secretion (exocytosis) by indirectly repressing Granulin/Slp4 via the transcription factor onecut-2. Another prominent target gene for miR-124 a is Fox A2 (forkhead box protein A2), a TF with several roles, namely beta-cell differentiation, glucose metabolism and insulin secretion. Silencing of miR-124 a in beta-cells causes a decrease in Fox A2 levels and its downstream target genes including PDX-1 (pancreatic and duodenal homeobox 1), Kir6. 2 (inwardly rectifying potassium channel), and Sur-1 (sulfonylurea receptor). Goto-Kakizaki (GK) rats, a non-obese model for Type 2 diabetes, are identified with miR-29 a and b. miR-29 is reported to be up-regulated in insulin target tissues (muscle, fat, and liver) of diabetic rats effecting insulin resistance\textsuperscript{[36]}.

The normal healthy beta cell perpetually adapts to a sustained stimulation by glucose for the synthesis of insulin. How is this life-long association of feedback mechanism maintained need to be understood to evaluate the insulin deficiency among diabetic patients? The network of micro RNAs may possibly provide a clue as they regulate gene expression through signal molecules. Assimilated glucose stimulates insulin gene promoter activity in beta cells through a cascade involving PI 3-kinase (phosphatidylinositol 3-kinase) which plays an important role in beta cell physiology. PI 3-kinase induces nuclear translocation of PDX-1 and the latter promotes the increase in insulin gene transcription. El Ouamari, et al.\textsuperscript{[34]} have shown that PI 3-kinase is involved in cell survival and proliferation. They performed transfection experiments using cell lines and insulinoma (INS-1E) cells were transfected with pmiR-375 expression vector. After 48 hours of transfection they found the reduction in cell number by $\sim 25\%$ and cell viability by $\sim 20\%$ and suggested that the enhanced expression of miR-375 facilitated cell viability and function\textsuperscript{[34]}. In yet another intriguing experiment they found that blocking miR-375 augmented PDK1 protein (pyruvate dehydrogenase kinase, isozyme 1) and the down regulation is shown in diabetic GK rats compared with healthy controls, and thus inferred that levels of miR-375 is regulated by glucose (Fig. 4), which confirms the molecular mechanism that upkeeps feedback information, a physiological phenomenon in vogue for the secretory function of beta cells. Further El Ouamari, et al.\textsuperscript{[34]} emphasized that miR-375 needs to be “prioritized” to enhance the islet function and to reverse beta cell failure. In future, therapy adopting microRNA-antagomiRs may target defined miRNAs that impede glucose homeostasis in diabetes.
Fig. 4 Up and down regulation of miR-375 on the insulin gene expression.

Fig. 5 miR-124a imprinting its neuronal functions on islets in improving beta cell secretory properties. E; Elevation; EC; Exocytotic components; L; Lowering.

4 GENETICS AND GENOME WIDE ASSOCIATION STUDIES IN TYPE-2 DIABETES

There is a worldwide epidemic of metabolic syndromes, viz., diabetes and obesity causing not only public health problems but also dwindling the family economy. Type 2 diabetes is a polygenic and heterogeneous disorder with several consequential metabolic anomalies. The advent of molecular tools and rapid accessibility of samples due to the crunching of the globe have provided us enormous inputs to assess the pathogenicity of hyperglycemia through multifactorial means. Studies pertaining to epidemiology, candidate gene, genetic linkage maps, pedigree analysis, etc., were in vogue up to the year 2000 and of course, paved the path by laying a strong foundation both at diagnosis and treatment and in research pursuits. Modern techniques comprising of high throughput technology for SNP genotyping, microarray for gene expression and protein analysis, ChIP on chip analysis, etc., are all gyrating research pursuits toward genome wide association (GWA) studies. HapMap project further complemented for the ongoing research in diabetes in genome-wide pattern of linkage disequilibrium. The recent reviews on the genetic basis of diabetes have elucidated the candidate genes and their target functions. A few prominent genes primarily involved in the viability, differentiation, metabolism, and cellular membrane physiology of pancreatic islets of beta cells have been shown in Tab. 2. As depicted in Fig. 6, the familial propensity plays a key role in the establishment of the glucose impaired metabolism. Ontogeny of beta cells, insulin expression, insulin sensitivity, glucose mediated synthesis of insulin, and insulin exocytosis are the target avenues for miRNAs, genes, and epigenetic factors to put their efforts. As genes follow the Mendelian pattern of inheritance, ample evidence has been piled up and deduced that there is a 40% risk among offspring if one of the parents is diabetic.
Tab. 2  Genes involved in Type 2 Diabetes

<table>
<thead>
<tr>
<th>S. N</th>
<th>Genes</th>
<th>Generic names of genes</th>
<th>Chromosomal location</th>
<th>Target functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gck</td>
<td>glucokinase</td>
<td>7p15</td>
<td>Glucose anabolism in hepatocytes</td>
</tr>
<tr>
<td>2</td>
<td>COX7A1</td>
<td>cytochrome oxidase gene</td>
<td>19q13.1</td>
<td>Mitochondrial dysfunction in skeletal muscle</td>
</tr>
<tr>
<td>3</td>
<td>PPARG</td>
<td>peroxisome proliferator-activated receptor gamma</td>
<td>3p25</td>
<td>Insulin sensitivity</td>
</tr>
<tr>
<td>4</td>
<td>CAPN10</td>
<td>calpin 10</td>
<td>2q37</td>
<td>Glucose transport</td>
</tr>
<tr>
<td>5</td>
<td>KCNJ11</td>
<td>potassium inwardly rectifying channel, subfamily J, number 11</td>
<td>11p15.1</td>
<td>Glucose sensing</td>
</tr>
<tr>
<td>6</td>
<td>TCF7L2</td>
<td>transcription factor 7 like-2</td>
<td>10q25</td>
<td>Pancreatic islet dev.</td>
</tr>
<tr>
<td>7</td>
<td>CDKAL1</td>
<td>DDHK5 regulatory subunit associated protein-1-like-1</td>
<td>6p22.3</td>
<td>CDK5 inhibition &amp; decrease insulin secretion</td>
</tr>
<tr>
<td>8</td>
<td>CDKX2A/V</td>
<td>cyclin-dependent kinase inhibitor 2A/B</td>
<td>9p21</td>
<td>Inhibits CDK4</td>
</tr>
<tr>
<td>9</td>
<td>HHEX/IDE</td>
<td>haematopoietically expressed homebox/Insulin degrading enzyme</td>
<td>10q23-25</td>
<td>Transportational repression in pancreatic development</td>
</tr>
<tr>
<td>10</td>
<td>SLC30A8</td>
<td>solute carrier molecule 30 member 8</td>
<td>8q24</td>
<td>Glucose induced insulin secretion</td>
</tr>
<tr>
<td>11</td>
<td>IGF2BP2</td>
<td>insulin-like growth factor 2 mRNA binding protein 2</td>
<td>3q27</td>
<td>Binds to 5’ UTR of IGF2 mRNA</td>
</tr>
<tr>
<td>12</td>
<td>FTO</td>
<td>fat mass and obesity associated</td>
<td>16q12.2</td>
<td>Adiposity</td>
</tr>
<tr>
<td>13</td>
<td>PDX1</td>
<td>pancreatic duodenal homebox</td>
<td>13q12</td>
<td>B-cell TF</td>
</tr>
</tbody>
</table>

Fig. 6  Flow chart representing multifactorial causes for the onset of hyperglycemia. DR: Down regulation; Risk factors: 1. Ageing; 2. Lifestyle; 3. Obesity.

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