Epigenetics in formation, function, and failure of the endocrine pancreas

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ABSTRACT

Background: Epigenetics, in the broadest sense, governs all aspects of the life of any multicellular organism, as it controls how differentiated cells arrive at their unique phenotype during development and differentiation, despite having a uniform (with some exceptions such as T-cells and germ cells) genetic make-up. The endocrine pancreas is no exception. Transcriptional regulators and epigenetic modifiers shape the differentiation of the five major endocrine cell types from their common precursor in the fetal pancreatic bud. Beyond their role in cell differentiation, interactions of the organism with the environment are also often encoded into permanent or semi-permanent epigenetic marks and affect cellular behavior and organismal health. Epigenetics is defined as any heritable — at least through one mitotic cell division — change in phenotype or trait that is not the result of a change in genomic DNA sequence, and it forms the basis that mediates the environmental impact on diabetes susceptibility and islet function.

Scope of review: We will summarize the impact of epigenetic regulation on islet cell development, maturation, function, and pathophysiology. We will briefly recapitulate the major epigenetic marks and their relationship to gene activity, and outline novel strategies to employ targeted epigenetic modifications as a tool to improve islet cell function.

Major conclusions: The improved understanding of the epigenetic underpinnings of islet cell differentiation, function and breakdown, as well as the development of innovative tools for their manipulation, is key to islet cell biology and the discovery of novel approaches to therapies for islet cell failure.

Keywords Endocrine pancreas; Islet cells; DNA methylation; Histone marks; Epigenetics

1. INTRODUCTION

All cells in a multicellular organism — excluding cell types such as B- and T-cells that undergo genomic rearrangements at specific sites to increase the body’s antibody and T-cell receptor diversity — contain an identical set of genomic instructions faithfully reproduced through many cycles of cell division from the zygote to the mature organism. It follows that diversity between cellular phenotypes and organ function must result from carefully orchestrated regulatory mechanisms that reinterpret the genomic DNA sequence, depending on cues such as developmental morphogens, cell position, hormonal milieu, diurnal cues, and environmental factors to activate and repress specific gene sets within each cell type. While most changes in gene activation status are acute and thus more properly thought of as direct control of gene expression, alterations in the gene activation status that are maintained through at least one mitotic cell division are considered ‘heritable’ and thus categorized as ‘epigenetic’ [1]. The Greek word ‘epi’ (επί) means ‘above, over’, thus indicating that epigenetic modifications control biological processes, from gene transcription to complex metabolic phenotypes, without altering the DNA sequence itself.

A striking example of epigenetic control is seen in the hundred or so autosomal loci in the human genome that are expressed in a ‘parent-of-origin’ specific pattern, meaning some are actively transcribed only on the maternally inherited chromosome while others are only transcribed from the paternal chromosome. This process is termed ‘imprinting’ and is mediated by specific patterns of DNA methylation [reviewed in [2]]. Defects in imprinting reveal the crucial role of this type of epigenetic regulation in the development and function of specific tissues. For example, imprinting affects islet cell function, as seen in Beckwith–Wiedemann syndrome, a severe organ overgrowth disorder caused by aberrant gene expression from an imprinted gene cluster located on chromosome 11 [reviewed in [3]]. This disease can result from either specific mutations within the imprinting control regions or from paternal disomy for this chromosomal region. As a result, expression of the tumor suppressor p57 (encoded by CDKN1C) is lost, as it is only transcribed from the maternal chromosome, resulting in impaired cell cycle control. In about half of the Beckwith–Wiedemann patient population, the resulting excess of β-cell mass results in hyperinsulinemia and hypoglycemia [4].

Another imprinted locus affecting insulin secretion is Transient Neonatal Diabetes Mellitus (TNDM). Neonates with TNDM present with hyperinsulinemia, which resolves by three months of age [5]. This disease can result from paternal hetero- or isodisomy or from aberrant methylation of the maternal allele, suggesting that overexpression of...
genes within this locus is responsible for the observed phenotype. ZAC (Zinc finger protein that regulates apoptosis and cell cycle) and the non-coding RNA HYMAI (hydatidiform mole-associated and imprinted region) are genes located in the TNDM locus, and murine dual transgenic overexpression recapitulates the human phenotype [6]. Beckwith–Wiedemann syndrome and TNDM thus represent striking examples of the impact of epigenetic control on islet growth and function.

On the molecular level, epigenetic states are ‘encoded’ both by DNA methylation, specifically cytosine methylation of palindromic CpG sequences, and by a multitude of histone modifications, which collectively determine chromatin compaction and accessibility of the transcriptional machinery to the more than 20,000 genes in the mammalian genome [7–19]. Frequently, non-coding RNAs such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are also considered to be epigenetic modifiers, as they affect final cellular steady-state mRNA and protein concentrations at the level of translation or mRNA stability, although strictly speaking, the expression of these non-coding RNAs themselves is under epigenetic and genetic control [20–23].

Epigenetic mechanisms affect organisational function on multiple timescales: transgenerationally, such as the aforementioned imprinting; within an organism to facilitate permanent or semi-permanent changes to mediate interactions between the environment and the genome [24,25]; or just a few days, e.g. recurrent cellular differentiation programs such as the generation of intestinal epithelial cells [26]. A unique feature of epigenetic modifications is that they permit adaptation to changing metabolic states while maintaining cellular function and can thus be both flexible and stable. As introduced above, the example of Beckwith–Wiedemann syndrome, epigenetic dysregulation can have dramatic pathophysiological consequences on islet cells and glucose homeostasis (see Table 1 for epigenetic modifiers with known roles in islet development and function).

2. MAJOR CLASSES OF EPIGENETIC MODIFICATIONS

2.1. Histone modifications

Genomic material in the cell is packaged into chromatin, which is a DNA-protein complex consisting of linear DNA wrapped around a histone core. A nucleosome is a unit of chromatin defined by 147 base pairs of DNA wound around the histone core, which includes four dimers each of the histones H2A, H2B, H3, and H4. The N-terminal tails of these histones can be covalently modified, which alters chromatin compaction and recruits transcriptional regulators to modulate gene expression in a multitude of ways [27,28]. Among the modifications to histones are acetylation of lysine and arginine residues, ubiquitination and sumoylation of lysines, and serine and threonine phosphorylation. Genome-wide maps for many of these modifications have been obtained through a technology termed ‘ChIP-Seq’, or chromatin immunoprecipitation followed by high throughput sequencing. For this assay, antibodies directed against specific histone modifications are employed to enrich for those chromatin fragments occupied by histones bearing these marks, and the precipitated DNA is purified and sequenced. Alignment of these sequenced fragments then reveals chromatin regions that are occupied by modified histones of interest. By mapping specific histone marks and the binding profiles of major islet transcription factors, Pasquali and colleagues were able to compile genome-wide maps of distinct chromatin states in human islets, from promoters to active enhancers [29]. Studies such as these have revealed that major chromatin states can be defined by just a handful of histone marks. Activating marks include the trimethylation of the fourth lysine on histone 3 (H3K4me3), which is associated with active promoters at genes enriched in CpG islands (see below), and acetylation of the 27th lysine of histone H3 (H3K27Ac), which is a mark of active enhancers, whereas repressed chromatin states are often marked by H3K27me3 [30]. Interestingly, combinations of specific histone modifications can also be relevant, such as in the case of a ‘bivalent’ chromatin state characterized by the presence of both the ‘repressive’ H3K27me3 and the ‘activating’ H3K4me3 mark at the same gene, a phenomenon first found in embryonic stem cells [31]. It is thought that this bivalent state facilitates the subsequent differentiation process, because genes will become active as soon as the H3K27me3 mark is removed from promoters or stably repressed if the H3K4me3 mark is erased. Together, histone modifications are reflected in transcriptional activation or chromatin compaction.

Chromatin compaction itself regulates accessibility of genetic loci to DNA-binding proteins, and is regulated, in turn, by the action of these sequence-specific transcription factors, especially the so-called ‘pioneer factors’ that can contact their binding sites even when nucleosome-bound within inactive chromatin regions [32]. There are multiple methodologies available for the study of chromatin accessibility, and one of the first systematic applications of these techniques to islet biology was published by Gaulton and colleagues in 2010, who employed “FAIRE-Seq” (formaldehyde-assisted isolation of regulatory elements followed by high-throughput sequencing) to map open chromatin regions in whole human islets [33]. They identified 340 genes with islet-selective open chromatin regions, among them, not surprisingly, the β-cell expressed PDX1, SLC30A8, and NKX6.1 loci, and found that open chromatin regions are enriched for SNPs associated with the genetic risk for type 2 diabetes. More recently, a much-improved technology, termed ‘ATACseq’, for ‘Assay for Transposase-Accessible Chromatin with high throughput sequencing’, became available to map open chromatin with much higher resolution [34,35]. ATACseq utilizes the hyperactive T5 transposase, which is used to simultaneously cut DNA and ligate adaptors for sequencing [36]. The Greenleaf group, recognizing that the transposase could only access naked or near-naked DNA, adapted this technology to use on intact open chromatin, which was then sequenced, while closed chromatin was inaccessible [34,35]. This approach has since been used successfully to provide detailed maps of accessible chromatin in sorted human ɑ- and β-cells, which will greatly facilitate the functional characterization of diabetes-associated genetic variants and aid in the integration of global transcription regulatory networks [37].

| Enzyme | Mechanism of action                                    | Effect on transcription | Effect of deletion on pancreas or islets in mice |
|--------|--------------------------------------------------------|------------------------|-----------------------------------------------|
| BrG1   | Part of SW/SNF complex; nucleosome remodeling; in the pancreas a Pdx1 coactivator | Silencing or activating | Hypoplastic pancreas when deleted early        |
| Dicer  | ncRNA processing                                      | Silencing or activating | Degrannulation and loss of β-cells            |
| Dnmt1  | Maintenance of CpG methylation                        | Usually silencing      | Hypoplastic pancreas when deleted early        |
| Dnmt3  | De novo CpG methylation                               | Usually silencing      | Dysregulation of glucose-stimulated insulin secretion |
| EzH2   | Part of PRC2, methylation of lysine 27 on H3 to H3K27  | Silencing              | Increased number of endocrine progenitors and mature β-cells |
2.2. DNA methylation

The second major epigenetic modification to our genomes occurs through methylation of nuclear DNA on specific residues, specifically at the C5 position of cytosines to create 5-methylcytosine. This modification occurs almost exclusively in the context of CpG dinucleotides in non-neuronal cells, which through their palindromic nature provide a convenient way to maintain the methylated state of a specific genomic region through mitotic cell divisions. This is so because once a DNA strand is replicated during S-phase, the enzyme DNA methyltransferase 1 (DNMT1) recognizes the now half-methylated sites and methylates the opposite cytosine to restore the original state utilizing S-adenosyl-methionine as the methyl donor (Figure 1). Thus, DNA methylation is the only epigenetic mark for which we understand inheritance through DNA replication. Most parent DNA methylation marks are erased during early embryogenesis and then reestablished and subsequently maintained in a cell type-specific manner. With the exception of the aforementioned imprinting diseases, which are caused by improper DNA methylation status, DNA methylation was considered for a long time to be a ‘boring’ epigenetic mark, because of its apparently invariant nature once established.

However, this all changed with the discovery in 2009 of an enzymatic pathway for targeted removal of DNA methylation (Figure 1). Members of the ‘Ten-eleven translocation’ or ‘Tet’ gene family were shown to be able to catalyze oxidation of 5mC to 5-hydroxymethylcytosine (5hmC) [38,39], which provides the starting point for two non-mutually exclusive mechanisms leading to demethylation of specific CpG’s. In the first model, the Tet enzymes can oxidize 5hmC further to 5-formylcytosine (5fC) and then 5-carboxylcytosine (5caC), although at reduced efficacy compared to oxidation from 5mC to 5hmC [40] (Figure 1). Following further enzymatic steps, 5caC bases can eventually be replaced by un-modified cytosines through the base excision repair mechanism [41]. Second, and perhaps more likely to matter in vivo, hydroxymethylation of specific cytosines results in their passive but targeted demethylation in replicating cells, because the maintenance DNA methyltransferases do not recognize hemimethylated 5 hmC nucleotides as a substrate following DNA replication during S-phase, resulting in an unmethylated newly synthesized strand (Figure 1). Regardless of the molecular mechanism, hydroxymethylation effected by the Tet enzymes at specific loci presents a pathway to decrease DNA methylation even for fully committed cell lineages. Around the same time as the discovery of the role of Tet enzymes in de-methylation, determining CpG methylation and hydroxymethylation genome-wide became feasible [42–44], quickly establishing that while the fully methylated state of repetitive elements is invariant, methylation levels at promoters and enhancers can be highly divergent between different tissues, and even dynamic within the same cell type, for instance during the aging process [45]. DNA methylation frequently represses transcription and causes gene silencing [6], although it was shown more recently that, on occasion, DNA methylation correlates with gene activation [46]. While in this review, we are focusing on the context of gene regulation and imprinting introduced above, DNA methylation is also critical for X chromosome inactivation in females, and to maintain genome integrity through inactivating transposable elements, many of which retain their ability to ‘jump,’ even once integrated into the human genome [47]. These divergent roles are reflected in the global landscape of DNA methylation, where large stretches of repetitive sequences, including those of transposable elements, are fully methylated, while gene promoters are frequently unmethylated.

The human genome contains about 30 million CpG dinucleotides, constituting approximately 1% of the haploid genome, much less than would be expected by chance, indicating that CpGs were actively selected against during evolution. While the majority of CpGs are dispersed sparsely throughout the genome, a small fraction (1–2%) is clustered into dense arrays termed ‘CpG islands’ (CGIs). CGIs are defined as genomic regions with more than 50% CpG content over a span greater than 500 base-pairs in length [48]. Strikingly, the majority of CGIs occur near RNA polymerase II promoters, suggesting a direct impact on gene transcription depending on their methylation status. CGI methylation can directly regulate gene expression by determining whether or not the basal transcriptional machinery can bind to a gene promoter. Most CpGs in the genome are highly methylated, and, as introduced above, this level of methylation ensures genomic stability by silencing transposable elements. In contrast, gene promoters and distal regulatory elements such as enhancers are often lowly and variably methylated [45,49,50].

2.3. Non-coding RNAs

A third type of epigenetic regulators are non-coding RNAs (ncRNAs). An interest in ncRNAs began in the 1990s. The long non-coding RNA (lncRNA) Xist, associated with the inactive X chromosome [51], and H19, associated with the imprinted IGF2-locus [52], were first
Mature miRNAs are 21—25 nucleotide single-stranded molecules with high sequence homology to the 3’ UTR of one or—more commonly—multiple protein-encoding RNAs. In mammals, they are processed by cleavage of longer double-stranded RNA precursors by the ribonuclease Dicer [60]. They bind their targets in an RNA-induced silencing complex (RISC) complex that includes an Argonaute protein, the enzyme that cleaves miRNA targets (reviewed in [61]). Higher homology miRNAs are targeted for immediate degradation, while for miRNA with lower sequence homology to miRNAs translation is blocked, leading to de-adenylation and ultimate degradation of the mRNA [59].

IncRNAs are defined as having a length of greater than 200 nucleotides, but they are often much longer. They are usually shorter than mRNA, but like mRNA are spliced and polyadenylated. Unlike miRNAs, IncRNAs are enriched in the nuclear fraction of the cell. IncRNAs can be divided into multiple classes [62]. These include: (1) long intergenic ncRNAs (lincRNAs) that do not overlap with any coding gene; (2) antisense IncRNAs; (3) pseudogenes; (4) long intronic ncRNAs; and (5) promoter- and enhancer-associated transcripts. These disparate IncRNAs function through a variety of mechanisms (Figure 2), and IncRNAs can both activate and repress gene expression. IncRNAs such as Xist act as scaffolds and bind both proteins that direct them to specific sites within the genome as well as the chromatin remodeling complexes that either silence or activate gene transcription [63]. At least in the case of Xist, spreading and silencing occurs only in cis, i.e. only on one of the two X-chromosomes, and seems to be controlled by proximity, explaining the major differences in Xist occupancy between the active and inactive X chromosomes [64]. Other ways IncRNAs can influence gene expression include mediating enhancer/promoter looping [65], repression of gene expression by antisense transcription [66], and stabilization/destabilization of mRNA by hybridization [67].

3. EPIGENETIC IMPACT ON ISLET CELL DEVELOPMENT

3.1. Epigenetic modifiers contribute to pancreas development

The pancreas is derived from the foregut endoderm, from which the liver, lung, esophagus, and stomach also arise. Cells in the foregut endoderm have to “decide” which path of differentiation to follow, and epigenetic processes help to shape this decision. Thus, when Xu and colleagues mapped key chromatic marks in pancreatic versus hepatic cell fate choices, they found that marks of active transcription (histone H3 acetylation) and polycomb-complex mediated repression (H3K27me3) were divergent between genes fated to be expressed in the pancreas versus the liver [68]. Consequently, genetic ablation of the enzymes responsible for these chromatin marks led to a partial redistribution of cells between the two primordia. Mouse or human embryonic stem cells can be differentiated into foregut endoderm, multipotent pancreatic progenitors, and even immature endocrine cells in vitro using precisely defined growth factors and other medium additives [69,70]. This paradigm by and large recapitulates the sequence of gene activation and repression events that occur during pancreatogenesis in vivo. For example, Wang and colleagues found that the FOXA transcription factors bind their enhancer targets as early as the foregut endoderm stage, while the pancreas-enriched transcription factor PDX1 occupies only a subset of enhancers once the embryonic stem cells are induced to differentiate towards the pancreatic lineage. At this later stage of pancreatic differentiation, FOXA binding at liver-specific enhancers is lost but maintained at pancreas-specific enhancers, and the chromatin at the latter changes from a ‘poised’ to an

characterized during that decade, and RNA interference through microRNAs (miRNAs) was discovered around the same time [53–55]. In recent years, there has been an explosion of knowledge and publication about the transcription, processing, and function of ncRNAs (for recent reviews see [56–59]).
‘active’ state, at least as reflected in specific histone modifications [71]. These observations in ES cell culture recapitulate earlier findings using mouse models that identified the Foxa and Pdx1 genes as essential to pancreas development [72–74]. Direct evidence for the critical role of epigenetic processes in pancreas development comes from multiple studies in which key epigenetic enzymes were removed in the developing pancreas using Cre/loxP-mediated cell type-specific gene ablation. Thus, without the DNA methyltransferase Dnmt1, pancreas development is halted due to inappropriate activation of the p53 locus [75]. Likewise, a hypoplastic pancreas results when a critical component of the SWI/SNF nucleosomal remodeling complex, Brg1, is ablated in the pancreas primordium, at least in part due its role as a Pdx1 co-activator [76]. In addition, pancreas-wide embryonic deletion of the miRNA-processing ribonuclease Dicer results in a hypoplastic pancreas, likely due to alterations in Notch signaling pathway components [77]. It is still unsurprising that complete ablation of these global regulators of the epigenome results in such dramatic phenotypes, but they still serve as a powerful confirmation of the concept that without the interplay of lineage-specific transcription factors with epigenetic enzymes, normal development cannot occur.

### 3.2. Differentiation of the endocrine cells types is controlled by epigenetic enzymes

Key evidence for the impact of epigenetic modifying enzymes comes from the use of specific inhibitors and various gene ablation models in the mouse. For instance, when explants of the embryonic pancreas anlage are cultured in the presence of HDAC (histone deacetylase) inhibitors, which would be expected to prevent gene silencing, multipotent progenitor cells produce an excess of endocrine cells at the expense of the exocrine pancreas, resulting in increases in both the β- and δ-cell populations [78]. As would be expected from these results, overexpression of HDAC enzymes led to decreased numbers of β- and δ-cells [79].

A key mediator of gene silencing at the chromatin level is the ‘Polycomb repressive complex’, named after a mutant Drosophila melanogaster fly. During early development, Polycomb group genes function in the silencing of Hox gene clusters in organisms ranging from flies to humans. The Polycomb repressive complex 2 (PRC2) acts by methylating lysine 27 of histone H3 to H3K27me3. This modification is followed by binding of the PRC1 complex, which ubiquitylates histone 2A (H2A), blocking the action of the histone 3 lysine 4 (H3K4)-methylating enzymes needed to achieve an active promoter state [80]. During differentiation of stem cells in vitro, transcriptional regulators tended to lose H3K27 trimethylation, while those that gained H3K27 trimethylation tend to be involved in cell function and morphology. When the PRC2 component Ezh2, necessary for the gene silencing process, was deleted in the pancreas anlage, the number of endocrine progenitors and hence mature β-cells was increased [81], which is consistent with the results from the stem cell analyses, since endocrine cells are among the first cells to differentiate within the pancreas.

### 3.3. Maintaining mature endocrine cell function and islet cell identity by epigenetic means

The major endocrine cell types, i.e. insulin-producing β-cells, glucagon-producing α-cells and somatostatin-secreting δ-cells, are closely related in terms of embryonic lineage and share common functional properties in terms of stimulus-secretion coupling, which is reflected in overall similar gene expression profiles [37,82]. Nevertheless, specific differences in gene activation programs need to be established and maintained to assure the unique functions of each cell type throughout life. This cell-type discrimination is accomplished by a highly complex network of DNA-binding transcription factors, whose individual impact has been delineated in great detail over the past 20 years through gene ablation studies in the mouse [72–74,83–85]. One particularly intriguing example, which also highlights the coordinated action of DNA-binding transcription factors with chromatin modifying enzymes, is the α-cell transcription factor Arx, or Aristalrelated homeobox. This DNA-binding protein is required for α-cell maintenance [94,95], but it also needs to be repressed in β-cells to prevent their transdifferentiation into glucagon-producing cells [96]. Arx silencing in β-cells is accomplished, at least in part, by binding of the transcription factor Nkx2.2 to the Arx promoter, followed by recruitment of the DNA methyltransferase Dnmt3, increased CpG methylation, and binding by MeCP2, a methylated DNA binding protein, to establish and maintain a fully repressed state [97,98]. Consequently, genetic ablation of the genes that establish DNA methylation at the Arx locus in β-cells causes transdifferentiation of the mutant cells towards the α-cell phenotype.

In addition to the manipulation of the enzymes that mediate epigenetic modifications described above, genome-wide studies of chromatin marks and open chromatin states are contributing to elucidate the epigenetic landscape of islet endocrine cell subtypes [29,37,99,100]. Given the remarkable plasticity of the endocrine cell types [94,96,101], it is quite satisfying that major histone marks such as the activating H3K4me3 and repressive H3K27me3 histone modifications are similar between human α- and β-cells, while a more dissimilar pattern is observed in the more distantly related exocrine pancreas [100]. Confirmation of the likeness of chromatin states between α- and β-cells was recently obtained by ATACseq analysis [37]. The remarkable cellular plasticity of α-cells as seen in mouse gene and cell ablation models finds its corollary in the fact that human α-cells exhibit many more ‘bivalently’ marked loci than α-cells. Bivalency refers to the simultaneous presence of activating H3K4me3 and repressive H3K27me3 histone modifications at the same promoter, an occurrence first observed in genes that need to be rapidly activated following differentiation from embryonic stem cells [31]. Non-coding RNAs help to maintain the identity and function of islet cells types. One of the first RNAs shown to have a role in pancreas function was miR-375 [102]. Its overexpression suppressed insulin secretion, and it repression conversely enhanced insulin secretion. Mice completely lacking mir-375 display impaired glucose homeostasis, increased α-cell mass, and decreased β-cell mass due to reduced proliferation [103]. Later, a wider role of miRNAs in β-cell function was discovered with a deletion of Dicer within the β-cell domain. These mice lose insulin expression, and the degranulated β-cells slowly disappear from the pancreas [104,105]. An appreciation of the importance of lncRNAs to islet biology has taken longer to develop than that for miRNAs. In 2012, Jorge Ferrer’s group reported the presence of >1,000 islet-specific lncRNAs in mice and examined the expression of IncRNAs during directed differentiation of human ES cells to insulin-producing cells [106]. Moreover, many IncRNAs were differentially regulated in islets from donors with type 2 diabetes compared to controls. In addition, deletion of the IncRNA termed ‘β-cell long intergenic non-coding RNA’ (lincrn) in mice resulted in a reduction of β-cells with a concomitant increase in δ-cells along with impaired glucose homeostasis [107].

A key functional property of the β-cell is its ability to sense a dynamic range of physiological glucose concentrations and react with a graded insulin secretory response. Central to this unique property is the near-exclusive expression of glucokinase, with a high Km for glucose (around 8 mM in humans) [108,109], as opposed to hexokinase, which
is active in neurons, skeletal muscle cells, etc. Because of the very low $K_m$ of hexokinase for glucose, it essentially always operates at $V_{max}$. In addition, glucokinase displays cooperativity, with a Hill-coefficient of 1.7, resulting in an inflection point in the glucose response curve of 3.9 mM, which enables it to function as the glucose sensor and to maintain the set-point for blood glucose of 5 mM [108–110]. Because of the low $K_m$ for glucose of hexokinase, it is critical that the β-cell does not activate transcription of HK1, the gene which encodes it, as any significant expression of this enzyme would shift the glucose response curve to the left, hence causing hypoglycemia. This consequence has been seen perhaps most strikingly in humans with activating $HK1$ promoter mutations which lead to congenital hyperinsulinism in children due to inappropriate expression of Hexokinase I [111]. Indeed, multiple enzymes and transporters exist, the expression of which will interfere with β-cell function; this fact has led to the concept that the corresponding genes are specifically repressed, or ‘disallowed’ in β-cells [110,112–116]. Multiple mouse models support a role for epigenetics in regulating these disallowed genes. In mice, deletion of the de novo DNA methyltransferase Dnmt3a resulted in promoter hypomethylation and elevated gene expression at Hk1 and Lactase Dehydrogenase A (Ldhα), another protein normally suppressed in mature β-cells [117]. Furthermore, Dicer inactivation in adult β-cells led to the same degranulation of β-cells observed when the gene was deleted developmentally and was accompanied by an upregulation of six normally silent genes [104,105,118].

Non-uniform insulin secretion and function by β-cells is a concept that has been revisited over the years [119–121]. New single-cell assays have provided multiple tools for investigating these different populations, including single-cell RNAseq [122–124], mass cytometry [125], single-cell calcium imaging [45,126], and the ability to delineate β-cell subpopulations on the basis of cell-surface antigens [127]. It is currently unclear whether this heterogeneity reflects different subgroups of β-cells, or merely temporary subpopulations that are cycling between states. Whichever is the case, epigenetics likely plays a role in the regulation of these subgroups. However, state-of-the-art single-cell techniques are unlikely to provide a satisfactory answer for any given gene, except for traditional bisulfite sequencing methods that assay one allele at a time [128]. Although single-cell ATACseq has already been reported [129,130], even for single-cell RNA-seq where most transcripts will have multiple copies, low or even moderate expression can lead to “drop-outs”, or false negatives, for expression of a certain gene in any given cell [131]. A single cell contains only two copies of a certain chromatin region or DNA sequence, resulting in either 0, 1, or 2 reads per cell. Thus, if in single-cell ATACseq a region is not sequenced, determining whether it is truly an area of inaccessible chromatin or if the sequence reads are missing by chance, is difficult. Computational biology tools that take into account thousands of genomic regions simultaneously will have to be devised in order to be able to determine if β-cell subtypes are reflected on the chromatin level.

4. EPIGENETICS AND HUMAN METABOLIC DISEASE

Given what we have learned about the impact of epigenetic factors for the development and maintenance of islet cell identity and function, it is not surprising that alterations in the epigenetic program can have severe pathophysiological consequences. In fact, multiple studies in rodent models and humans have shown that even in utero exposure can lead to multi-generational inheritance of metabolic disease (see below), which clearly has to be mediated by epigenetic processes, as there are no mutations in the DNA sequence evident in these processes. Likewise, the obvious effects of the modern lifestyle of limited physical activity and obesity have a dramatic effect on the incidence of type 2 diabetes in the Western and increasingly also the developing world. The worldwide increase in type 2 diabetes cannot be explained by an altered prevalence of disease risk-conferring mutations. While the precise molecular causality is much more difficult to prove for epigenetic events than for mutations to the DNA, an important contribution of an altered epigenome to T2D susceptibility is supported by multiple lines of evidence.

4.1. Intrauterine development and the transgenerational epigenetic inheritance of metabolic disease

The ‘Dutch hunger winter’ (‘hongerwinter’ in Dutch) is the most famous example of the effects of maternal diet to diabetes risk. At the end of World War II, Nazi occupiers imposed a strict diet on the population of the northwestern Netherlands, with daily rations as low as 600 kcal. Children of the mothers that were pregnant during this time period were not only born with low birth weight as expected, but also had a significantly higher risk of metabolic disease as adults [132]. A separate study following boys with known birth-weight until age 64 found that individuals with low birth weight had a higher incidence of impaired glucose tolerance and impaired β-cell function than others in the same cohort [133].

Rodent models offer the opportunity to study these phenomena in isolation and to find correlates of phenotypic changes in the epigenome. The effects of maternal nutritional status during pregnancy on the long-term metabolic health of offspring can be studied in models of intrauterine growth restriction (IUGR), either by providing very limited maternal nutrition or by performing uterine artery ligation to induce uteroplacental insufficiency [134,135]. While these paradigms of course affect multiple organ systems, in many cases the activity of crucial β-cell transcription factors such as Pdx1 was altered, evidently due to a modified epigenetic state [136–138]. In another paradigm, pregnant dams were fed isocaloric diets, but one group was protein-restricted. This alteration in macronutrient intake was sufficient to cause hypermethylation and decreased activation of the Hnf4α promoter, an effect that did not resolve as the offspring aged [138].

In the scenarios described thus far, the fetus itself is exposed to detrimental conditions that affect its metabolic health; thus, they are examples of the ‘developmental origins of health and disease’, or DOHAD, hypothesis [133]. Remarkably, though, in certain detrimental conditions effects are seen in the generation not directly exposed to the stimulus, resulting in transgenerational inheritance not mediated by changes to the DNA sequence.

4.2. Parental transmission of epigenetic risk for metabolic dysfunction

When considering the consequences of the DOHAD hypothesis, it is essential to be aware that aberrant epigenetic alterations can occur by exposure to detrimental conditions in utero, as exemplified by the Dutch hunger winter described above, or through inheritance of epigenetic effects to the next generation via molecular changes to the parental gametes. Finding the molecular evidence for transmission of epigenetic marks through parental gametes is particularly difficult since most epigenetic marks such as DNA methylation and histone modifications undergo near wholesale erasure during gametogenesis and again in the preimplantation embryo post-fertilization to establish and maintain cellular pluripotency. Thus, for true epigenetic transgenerational inheritance, environmental cues must be reflected in permanent epigenetic changes that are exempt from erasure. Therefore, it is essential to carefully consider the timing of environmental exposure to differentiate
multigenerational from transgenerational epigenetic effects. Thus, when a F1 fetus is exposed to a detrimental condition such as nutrient deprivation or maternal stress in utero, the gametes of that fetus (F2) will also experience the biochemical consequences of the exposure. Therefore, transmission of an epigenetic effect should only be considered to be transgenerational if its phenotypic consequences are also observed in the F3 generation (see Figure 3).

A worrisome example of detrimental environmental exposures that have multigenerational effects on β-cell function is parental exposure to endocrine disruptors. While malnutrition can be easily diagnosed and prevented at trivial costs, the effects of environmental toxins are mostly unknown and can take decades to be discovered. One such industrial chemical is Bisphenol A (BPA), which is among the most commonly produced chemicals in the world today, with an annual production of 4 million metric tons. BPA is used for the synthesis of epoxy resins and plastics, and finds its way into ubiquitous consumer goods such as water bottles. BPA and related compounds mimic the action of estrogens, and fetal exposure to BPA causes impaired glucose handling in rodents, which appears to be caused by altering the epigenetic state of β-cell expressed genes Pdx1 and Igf2, resulting in impaired insulin secretion [139,140]. Sperm of the F1 generation after BPA exposure exhibited similar changes to the DNA methylation state as the F0 generation, suggesting that in this case, and likely other, epigenetic transmission can occur via the male lineage [141]. Given the plethora of new chemical structures and xenobiotics being produced world-wide, it is extremely likely that many other detrimental effects of environmental toxins will be discovered in the future, a subset of which will have multigenerational and transgenerational effects.

When males are exposed to detrimental or diabetogenic conditions, the paternal contribution can be isolated to epigenetic information carried in sperm and observed in phenotypes of the F2 generation. Thus, when male rats where fed a chronic high fat diet (HFD), their female offspring showed glucose intolerance and reduced β-cell function that worsened with age, which clearly had to be the consequence of epigenetic changes carried in the sperm. In fact, many genes known to function in insulin exocytosis and β-cell survival were differentially expressed in β-cells of females born to an obese father compared to those of females that were offspring of a lean male [142]. Remarkably, new evidence is accumulating that these epigenetic effects on metabolic health are not confined to rodents, but can also occur in humans, as the sperm DNA methylome was found to be significantly different between lean and obese men [143], suggesting, though not proving, that a predisposition to obesity could be transmitted epigenetically.

4.3. The methylome is dynamic during ontogeny

When comparing genome-wide profiles and maps of various histone marks to those of DNA methylation among cell types and between individuals, it is quite clear that DNA methylation is the more uniform and stable mark. In fact, originally it was thought that once the methylome is established in the various cell lineages during early fetal development, the identical methylation pattern is maintained in each cell type, because the maintenance DNA methylase DNMT1, which is associated with the DNA replication fork, immediately remethylates the hemimethylated CpGs that result from DNA replication. As introduced above, this view is clearly an over-simplification, and the methylation state can be highly dynamic even within the same cell lineage, as shown by genome-wide methylation studies comparing, for instance, young and old β-cells [45]. The discovery of the Tet hydroxymethylation enzymes provided the molecular underpinnings for a targeted demethylation pathway (Figure 1).

Figure 3: Epigenetic inheritance can be multigenerational or transgenerational. A) Multigenerational inheritance refers to a change in a trait or phenotype in the F1 offspring of males or non-pregnant females (F0) exposed to a stimulus that impacts the epigenome without changing the DNA sequence. B) In the case of a pregnant female exposed to an environmental toxin, for instance, the F0 parent, the F1 fetus, and the F2 germline within the fetus are all exposed. Therefore, in this case, only if the F3 generation also shows an epigenetically altered phenotype does transgenerational inheritance occur.
Consequently, for many key β-cell factors, changes in the methylation status of their promoter have been determined and correlated with changes in gene activity and islet function. For instance, it was found that in islets from people with type 2 diabetes, the promoter of the key transcriptional coactivator PGC-1α (peroxisome proliferator activated receptor gamma coactivator-1 alpha) is hypermethylated compared to controls and also exhibits reduced steady-state transcript levels [144], providing a possible explanation for reduced mitochondrial function in diabetic β-cells. In a different study, increases in body mass index and hemoglobin A1c correlated with increased islet cell DNA methylation and reduced expression of the glucagon-like peptide 1 receptor, which suggests a reduced responsiveness of β-cells to incretins in obese and/or diabetic patients [145]. While it is impossible in these cross-sectional studies to prove that aberrant DNA methylation of any specific gene causes diabetes, they nevertheless suggest that impaired epigenetic regulation of islet cell genes could factor into disease development.

Today, it is possible to determine the DNA methylation status genomewide, either by ‘bisulfite sequencing’ or by array-based methodologies. While array-based techniques offer methylation analysis at lower costs than bisulfite sequencing, they only cover about 0.5% of all CpG sites in the genome and therefore can miss important changes to the methylome. Array-based methods were used to find 273 differentially methylated regions in whole islets when comparing the promoter methylation of five donors with type 2 diabetes to eleven controls [146]. However, genome-wide methylation analysis employed in a similar experiment with six controls and eight donors with type 2 discovered 25,820 differentially methylated regions [147]. Using this same technology, Avrahami and colleagues showed that the mouse β-cell methylome is dynamic with age, especially at distal enhancers, and that alterations frequently occur in genes that control β-cell function [45].

5. CONCLUSIONS

The interpretation of the genome—with a few exceptions the same in all our cell types—is essential to enable the development of multicellular organisms with a division of function among the multiple organ systems and cell types. The micro-organ of the pancreatic islet is no exception, and epigenetic regulators play key roles in endocrine cell development, differentiation, and function. While over the past 20 years, the focus of researchers world-wide has been on the determination of the function of individual epigenetic factors, and more recently, the genome-wide mapping of epigenetic marks with increasing breadth and depth of coverage, the future promises to allow for direct, targeted control of gene function through epigenetic control. In addition to broad-acting drugs, such as inhibitors of histone deacetylase, which can reduce the sensitivity of islets to apoptosis-inducing cytokines [148], current and future approaches will employ mutated TALE and CRISPR-Cas proteins tethered to epigenetic enzymes such as DNA methyltransferases, a combination which can target these modifiers to a single selected locus in the genome [149,150]. These approaches will thus enable control of islet cell function and proliferation without any changes to the DNA sequence. When combined with cell-type specific delivery systems, these methods will be able to elevate precision medicine for diabetes to a whole new level.

CONFLICT OF INTEREST

None declared.
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