Review

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Epigenetic modifications and long noncoding RNAs influence pancreas development and function

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Insulin-producing β cells within the pancreatic islet of Langerhans are responsible for maintaining glucose homeostasis; the loss or malfunction of β cells results in diabetes mellitus. Recent advances in cell purification strategies and sequencing technologies as well as novel molecular tools have revealed that epigenetic modifications and long noncoding RNAs (lncRNAs) represent an integral part of the transcriptional mechanisms regulating pancreas development and β cell function. Importantly, these findings have uncovered a new layer of gene regulation in the pancreas that can be exploited to enhance the restoration and/or repair of β cells to treat diabetes.

Pancreas development

The pancreas is a bifunctional organ with exocrine and endocrine tissues regulating digestive processes and glucose homeostasis, respectively. These functionally distinct tissues arise from a common pancreatic progenitor pool in two major waves of differentiation termed the primary and secondary transitions (Figure 1) [1]. The primary transition refers to the specification of pancreas progenitors within the foregut endoderm and requires the essential transcription factors pancreatic and duodenal homeobox 1 (Pdx1) and pancreatic transcription factor 1a (Ptf1a). The secondary transition refers to the stage where pancreatic progenitors expand and external signals direct cells toward endocrine versus exocrine fates. A critical phase of pancreatic islet differentiation is the activation of the basic helix–loop–helix transcription factor Neurogenin 3 (*Neurog3*) within a subset of pancreatic progenitor cells to specify the endocrine precursor pool; deletion of *Neurog3* results in the absence of endocrine cell development [2]. In the adult pancreas, there are four endocrine cell types that constitute the mature islet: insulin-producing β cells, glucagon-producing α cells, somatostatin-producing δ cells, and pancreatic polypeptide-producing PP cells. The cell fate determination of these four endocrine cell populations within the Neurog3^{+} cells depends on several additional transcription factors including Pdx1, Nkx2.2, Pax4, Pax6, Isl1, NeuroD1, Arx, and Nkx6.1 (reviewed in [3]). In this review we discuss recent advances in the characterization of epigenetic changes that occur in the specification of the pancreatic endocrine cells, with a special focus on β cells (summarized in Figure 1). We also discuss how epigenetics can play a role in the etiology and treatment of pancreas-related diseases and speculate on the putative role of lncRNAs in the maturation and function of β cells. These studies are primarily based on genetic models of murine pancreas development (Table 1), isolated rodent and human islets, and *in vitro* differentiated ES cells.

Epigenetic modifications during pancreas development

Endoderm differentiation

Cellular differentiation requires the establishment and maintenance of tissue-specific patterns of gene expression in response to extracellular signaling. As with many developing tissues, epigenetic modifications within endodermal cells are dynamically positioned or removed to regulate gene expression in response to developmental cues (Box 1). In particular, the promoters of lineage-determining factors are often enriched for epigenetic marks of both active and repressive chromatin (H3K4me3 and H3K27me3, respectively) in a bivalent, 'poised' state [4] that allows rapid activation during development. In support of this idea, genomic analyses of endoderm differentiated from human ES cells (hESCs) *in vitro* demonstrates that bivalent

Glossary

DNA methyltransferases (DNMTs): Catalyze the methylation of DNA, which, in mammals, occurs mainly on cytosines in CpG dinucleotides.
Enhancer of zeste 2 (EZH2): a histone methyltransferase member of Polycomb repressive complex 2 (PRC2) that mediates the methylation of histone 3 on lysine 4 (H3K4me3); associated with transcriptional repression.
Histone acetyltransferases (HATs): acetylate lysine residues of histones. Neutralize the negative charge of DNA, relaxing the chromatin structure and promoting transcription.
Histone deacetylases (HDACs): remove acetyl groups from lysine residues on histones, enabling chromatin to compact and causing transcriptional repression.
Histone demethylases (HDMs): remove methyl groups from histones.
Histone methyltransferases (HMTs): add methyl groups to lysine or arginine residues of histones.
KDM6: a family of HDMs that includes KDM6a and KDM6b. Remove methyl groups from lysine-27 di- and trimethylated on histone 3 (H3K27me2/3).
Polycomb repressive complex 1 (PRC1): monoubiquitylates histone H2A on lysine-119 (H2AK119ub1) and represses transcription.
Polycomb repressive complex 2 (PRC2): a complex of proteins with HMT activity. Primarily trimethylates histone 3 on lysine-27 (H3K27me3) and represses transcription.

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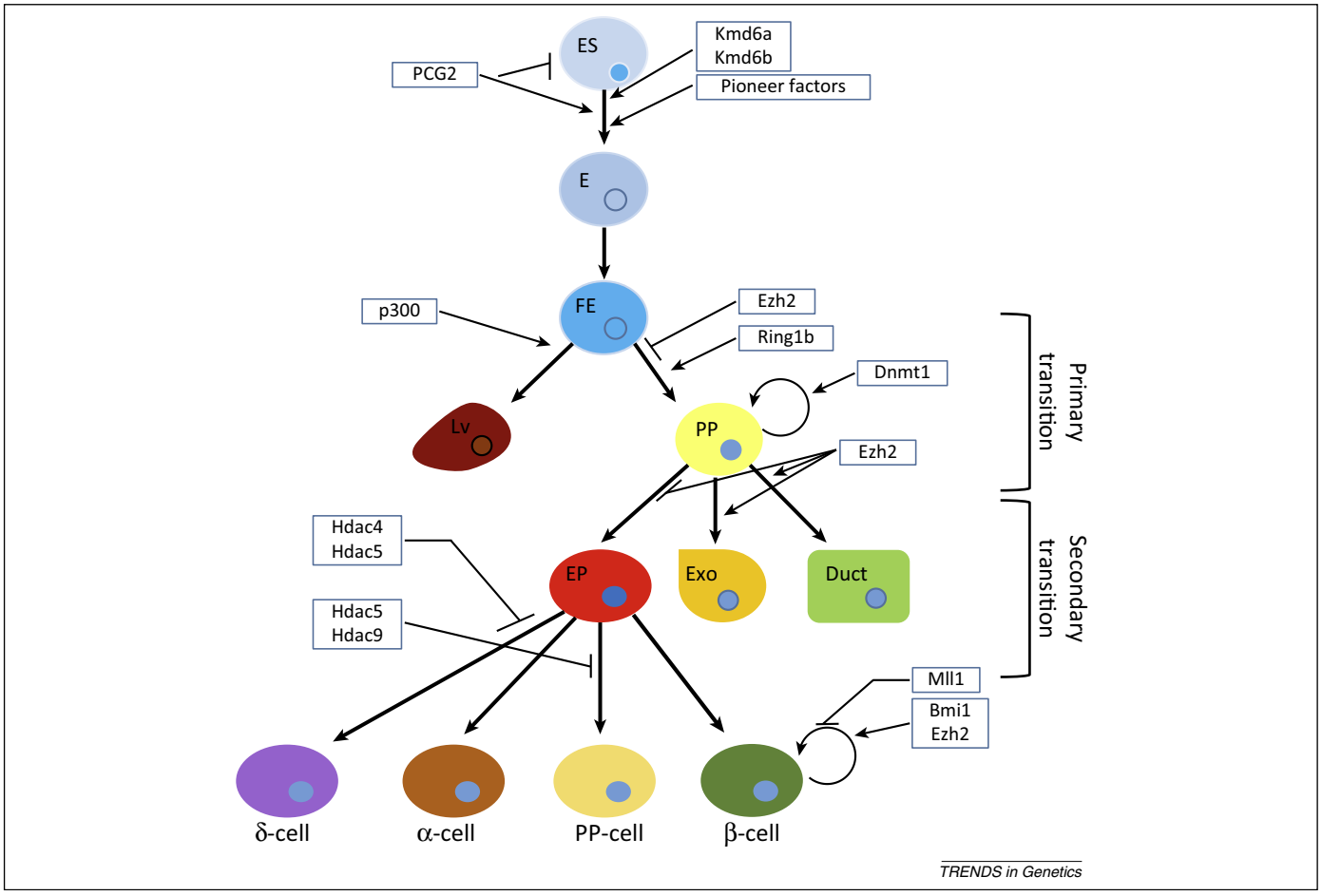

Figure 1. Epigenetics and pancreas development. Schematic representation of pancreas development with epigenetic modifiers involved in the process depicted in boxes. Abbreviations: E, endoderm; FE, foregut endoderm; Lv, liver; PP, pancreas progenitors; EP, endocrine progenitors; Exo, exocrine cell.

promoters are resolved to activate gene expression through depletion of H3K27me3 or to repress gene expression through *de novo* H3K27 methylation [5,6]. Consistently, H3K27me3 generated by Polycomb repressive complex 2 (PRC2) (see Glossary) is necessary for the repression of pluripotency factors in the differentiating hESCs and for the appropriate specification of endoderm lineages [7]. Furthermore, the histone H3K27me2/3 demethylases KDM6A and KDM6B are upregulated after endoderm induction in hESCs [8], whereas their knock down in hESCs significantly dysregulates WNT signaling and reduces the efficiency of endoderm specification [5,8]. *In vivo*, definitive endoderm specification relies on extracellular signaling of Nodal/ActivinA via Smad2/3 phosphorylation to activate lineage-specific transcription factors (for a review see [9]). Based on *in vitro* differentiation studies, it has been proposed that the resolution of bivalent marks may be due to an interaction between KDM6B and SMAD2/3 causing loss of the H3K27me3 repressive mark in SMAD target genes [6,10]. Consistently, the promoter of *Eomes*, a master regulator of endoderm induction [11,12], is demethylated through the recruitment of Kdm6b and Tbx3 to a regulatory region upstream of the *Eomes* transcriptional start site (TSS). This facilitates enhancer–promoter association and demethylation of H3K27me3 to promote *Eomes* expression [13].

In addition to dynamic changes in histone methylation states, a subset of active endodermal genes also has a distinct signature of histone modifications at their enhancers [14]. The most common modification is the deposition of H2A.Z (associated with gene activation), suggesting a mechanism by which the enhancers of lineage-determinant genes are primed to be responsive to endodermal transcription factors. Lineage determination can also be specified by the action of pioneer factors, which modify the chromatin to allow access of other cell-fate-specific transcription factors. Endoderm differentiation is known to be dependent on two pioneering transcription factors, Foxa2 and Gata4 (reviewed in [15]). Foxa2 and H2A.Z regulate nucleosome depletion and the activation of endodermal genes [16] and Gata4 is known to facilitate the acetylation of H3K27 mediated by the histone acetyltransferase p300 [17]. This suggests a potential mechanism by which endoderm specification is initiated by pioneer factors.

These studies and others provide evidence that chromatin remodeling mediated by histone methyltransferases (HMTs), histone demethylases (HDMs), and other histone modifications (Box 1) plays essential roles in endoderm specification. However, the timely regulation of chromatin modifications, the binding of transcription factors, and how these transcription factors are targeted to the appropriate chromatin domain remains elusive.