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Review

Epigenetic regulation of pancreas development and function

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ABSTRACT

Multiple signaling systems and transcription factor cascades control pancreas development and endocrine cell fate determination. Epigenetic processes contribute to the control of this transcriptional hierarchy, involving both histone modifications and DNA methylation. Here, we summarize recent advances in the field that demonstrate the importance of epigenetic regulation in pancreas development, β-cell proliferation, and cell fate choice. These breakthroughs were made using the phenotypic analysis of mice with mutations in genes that encode histone modifying enzymes and related proteins; by application of activators or inhibitors of the enzymes that acetylate or methylate histones to fetal pancreatic explants in culture; and by genomic approaches that determined the patterns of histone modifications and chromatin state genome-wide.

Contents

1. Introduction

Pancreas development and endocrine cell fate determination are controlled by precisely timed signaling events, discussed in detail in (Serup, in this issue), which determine the chronology of activation and repression of transcriptional networks [1,2]. The transcriptional hierarchy that regulates gene expression during development and disease is in part regulated by epigenetic processes, involving both histone and DNA modifications, which in turn facilitate or prevent recruitment of effectors protein complexes.

Epigenetic events were originally defined as those heritable changes to phenotype that occurred without altering the DNA sequence itself. This definition has been loosened in recent years to include control of gene expression by DNA methylation and histone modification, even when this is not heritable through mitosis. One example of the striking functional significance of epigenetic alterations is the silencing of tumor suppressor genes that can occur in cancer and which is mediated through DNA methylation and silencing of promoters. In mammalian cells, DNA methylation occurs on cytosines in the context of CpG dinucleotides at the 5 position to create 5-methylcytosine, and is mediated by methyltransferase enzymes encoded by three genes, *Dnmt1*, *Dnmt3a* and *Dnmt3b*. Over the past two decades, dozens of modifications to histones, including lysine (K) acetylation, lysine and arginine (R) methylation, serine (S) and threonine (T) phosphorylation, and lysine sumoylation and ubiquitination have been shown to affect gene expression in multiple ways.

Here we describe recent findings demonstrating the involvement of epigenetic mechanisms in pancreas development, post-natal regeneration of the insulin-producing β-cell, and preservation of lineage identity through cell divisions. These findings were obtained using three general approaches: (1) the phenotypic analysis of model organisms, chiefly mice, with mutations in one or several of the genes that encode histone modifying enzymes and related proteins; (2) the use of more or less specific activators

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or inhibitors of the enzymes that acetylate or methylate histones; and (3) genomic approaches that determine the patterns of histone modifications and chromatin state genome-wide, and make inferences about gene regulation by comparison to similar maps from other cell types.

2. Genetic analysis of mutations for histone modification enzymes in pancreas development and β-cell replication

Genome-wide location analysis of embryonic stem (ES) cells has identified a unique histone modification pattern, termed "bivalent domain," in which repressive histone marks, *i.e.* H3K27me3, and activating marks, *i.e.* H3K4me3, are present at the same location [3]. In general, the repressive mark is dominant, meaning the corresponding gene is silent in ES cells. Many of these bivalent domains were found at important developmental determination genes, which are thus 'poised' for rapid activation during ES cell differentiation by simple removal of the repressive mark.

Xu et al. [4] explored the possibility that such mechanisms exist also in multipotent cells of the developing endoderm, prior to the fate choice between liver and ventral pancreas identity. Chromatin immunoprecipitation (ChIP) analyses of multiple histone modifications of early liver and pancreas-specific genes from FACS-sorted endoderm cells (day E8.25) or hepatoblasts (day E9.5), showed that all hepatic lineage-specific genes are marked as 'silent' in endoderm cells as expected, while these liver-specific genes become marked as 'activated' in hepatoblasts. Two chromatin marks were found to be different in endoderm cells between liver- and pancreas-specific genes. Thus, H3K9acK14ac, associated with gene activation, was poorly represented in the regulatory elements of liver-specific genes such as *Alb1*, *Afp* and *Ttr*, but enriched in the regulatory elements of PDX1, an early pancreatic gene. Similarly, H3K27me3, associated with gene silencing, was also under-represented in 'liver elements', but enriched at the promoter of the PDX1 gene. In other words, in early endodermal cells, PDX1 was bivalently marked, while liver-specific genes carried no histone modification marks at all. However, following differentiation into hepatoblasts, H3K9acK14ac increased on liver-specific elements, while H3K27me3 remained low, whereas the PDX1 promoter remained hyperacetylated and enriched for H3K27me3, indicative of distinct chromatin states for the two types of genes.

Based on these findings, Xu and colleagues studied the enzymatic machinery underlying this differential state, by using gene-targeted mice heterozygous for P300 ($P300^{+/-}$), a histone acetyltransferase. ChIP analysis showed increased levels of histone acetylation in promoters of the early liver marker genes *Alb1*, *Afp* and *Ttr*, in wild type hepatoblasts compared to $P300^{+/-}$ hepatoblasts. In parallel, the expression of these early liver-specific genes was diminished in $P300^{+/-}$ hepatoblasts, whereas PDX1 expression was upregulated. These findings suggest that P300 is necessary for acetylation, and thus activation, of liver-specific regulatory elements, and that P300 modulates the cell fate choice between liver progenitors and pancreas progenitors.

Next, the authors showed, again using ChIP analysis, enrichment of Ezh2, a methyltransferase for H3K27me3 and a member of PRC2 (polycomb-repressive complex 2) at upstream regulatory elements in the PDX1 gene. Binding of Ezh2 was overlapping with H3K27me3, but absent from liver-specific regulatory elements in wild type endoderm cells. Following deletion of the Ezh2 allele in foregut endoderm using an Ezh2 conditional allele and the FoxA3-Cre transgene, embryos at E10 exhibited an expanded PDX1-positive ventral pancreas domain, accompanied by multiple bud-like structures, leading to an enlarged ventral pancreas at E11.5. This expansion of the pancreas occurred at the expense of liver development. In conclusion, Ezh2 normally promotes the liver program by restraining pancreatic commitment (see Fig. 1 for schematic summary).

During embryonic development, cell specification is achieved by activation and repression of transcription factors in response to inductive developmental signals. Transcriptional programs are somewhat plastic, and thus cellular fates can be "re-programmed" in extreme conditions. For example, misexpression of the α-cell-specific transcription factor Aristaless homeobox gene (*Arx*) in fetal β-cells causes β-cell to α-cell conversion [5]. Furthermore, Thorel and colleagues have shown that mice with over 90% reduction in β-cell mass are able to replenish some of the lost cells through up-regulation of β-cells transcription factors in α-cells, causing α to β-cell transdifferentiation [6]. However, the normal complement of functional β-cells was not restored in this model. Nevertheless, these examples of transdifferentiation point to a close developmental relationship between α and β-cells, and suggest that they exist in a similar epigenetic state.

Nkx2.2 is a homeodomain transcription factor required for pancreatic islet cell fate decisions [7]. In a recent study, Papizan and colleagues showed that in β-cells, Nkx2.2 is part of a repression complex, together with DNMT3a – a *de novo* DNA methyltransferase important for establishing methylation patterns during development [8], the groucho-related repressor Grg3, and the histone deacetylase HDAC1 [9]. To investigate the role of this complex in pancreatic islet development, they derived mice homozygous for a specific point mutation in the *tinman* (TN) domain of Nkx2.2 ($Nkx2.2^{TNmut/TNmut}$), which disrupts the interaction between NKx2.2 and Grg3. These mice develop hyperglycemia and do not survive beyond eight weeks of age. Mutant islets are smaller and contain fewer β-cells and more α-cells, presumed to have formed at the expense of the β-cells population. Interestingly, by the end of the gestation, the mutant mice present a distinct population of β-cells expressing the α-cell specific transcription factor *Arx*.

Based on the hypothesis that the mutation in the *Nkx2.2* TN domain is causing derepression of the *Arx* gene in β-cells, Papizan and colleagues permanently marked β-cells by genetic lineage tracing. This involved transgenic mice with an insulin promoter driven Cre recombinase (Ins:Cre) transgene combined with the Rosa26:LacZ reporter allele, which leads to permanent expression of β-galactosidase, the product of the bacterial LacZ gene, in all cells that express Cre recombinase and their descendants. Indeed, the authors found that a fraction of the β-galactosidase marked cells expressed *Arx*, demonstrating that β-cells had been reprogrammed towards an α-cell fate in the absence of fully functional *Nkx2.2*. Bisulfite analyses to determine the CpG methylation status at the *Arx* promoter showed that *Nkx2.2* occupies the hypermethylated *Arx* promoter in both α and β cells. However, the other members of the repressive complex, *i.e.* Grg3, HDAC1 and Dnmt3a, were found preferentially at the *Arx* promoter in β-cells. Taken together, this study demonstrates the role of *Nkx2.2* and *Dnmt3a* in recruiting a repressor complex to the *Arx* promoter in β-cells to maintain their identity. Although *Nkx2.2* is expressed and functions in both α and β-cells, its binding is epigenetically regulated to preferentially occupy the *Arx* promoter in β-cells. This differential binding is influenced by both the methylation state of the *Arx* promoter and by the DNA modifications induced by *Dnmt3a* (see Fig. 1).

In the post-natal pancreas, terminally differential β-cells preserve the potential to proliferate during maturation and in response to injury, in order to maintain glucose homeostasis [10–12]. Therefore, a tight regulatory system is needed to limit proliferation and to maintain cell identity after cell division. DNA methylation is one of the mechanisms that can ensure stable inheritance of repressed genes. Recently, Dhawan and colleagues [13] used β-cell specific ablation of the *Dnmt1* gene, a DNA methyltransferase that restores CpG methylation pattern after DNA replication in S-phase of the