Epigenetics mechanisms in renal development

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Abstract

Appreciation for the role of epigenetic modifications in the diagnosis and treatment of diseases is fast gaining attention. Treatment of chronic kidney disease stemming from diabetes or hypertension as well as Wilm's tumor will all profit from knowledge of the changes in the epigenomic landscapes. To do so it is essential to characterize the epigenomic modifiers and their modifications under normal physiological conditions. The transcription factor Pax2 was identified as a major epigenetic player in the early specification of the kidney. Notably, the progenitors of all nephrons that reside in the cap mesenchyme display a unique bivalent histone signature (expressing repressive epigenetic marks alongside activation marks) on lineage-specific genes. These cells are deemed poised for differentiation and commitment to the nephrogenic lineage. In response to the appropriate inducing signal, these genes lose their repressive histone marks, which allow for their expression in nascent nephron precursors. Such knowledge of the epigenetic landscape and the resultant cell fate or behavior in the developing kidney will greatly improve the overall success in designing regenerative strategies and tissue reprogramming methodologies from pluripotent cells.

Keywords

kidney development; histone code; epigenetics; nephrogenesis; nephron progenitors

Epigenetics

Epigenetics refers to mechanisms that prevail above and beyond the information encoded in the DNA sequence (the genetic code) in regulating gene expression. This is brought about by covalent modifications, not just to the nucleotides in the DNA, but also to the histone proteins that are closely associated with them. These modifications are heritable - although non-genetic in nature – and can be passed on during cellular divisions [1]. Epigenetic mechanisms are paramount to the development of metazoan organisms. Although the cells in these organisms have identical genotype, by virtue of epigenetic mechanisms, they diversify during the course of development to form highly specialized tissues and organ systems.

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Despite the heritable nature of epigenetic modifications they are reversible [2]. Covalent modifications such as methylation, acetylation, phosphorylation, sumoylation and ubiquitination together with the enzyme mediators of these modifications constitute the major players. The DNA within the nucleus is organized into a complex structure called chromatin. The chromatin is formed of a repeating structural unit: the nucleosome. Each nucleosome has a protein core formed of histone protein dimers (H2A, H2B, H3, and H4) wrapped by 146bp of double-stranded DNA. The tails of the histone proteins that extend out of the core are a hot bed for covalent modifications especially on the lysine (K) residues. Segments of chromatin bearing different modifications dynamically assume a "closed" (transcriptionally silent) or "open" (transcriptionally active) conformation, which allows the expression of lineage-specific genes in a spatial and temporal manner. The tightly packed, transcriptionally silent chromatin is called heterochromatin while the loosely packed, transcriptionally active chromatin is called euchromatin. Gene regulation at euchromatin regions is guided by epigenetic modifications to modular, cis-acting gene elements, e.g., promoters, enhancers, silencers, insulators, locus control regions. Apart from histone modifications, the methylation of cytosine nucleotides in the DNA itself, as well as non-coding RNAs is included as part and parcel of the epigenetic regulatory signature.

Epigenetic DNA modifiers

Several of the genetic polymorphisms related to complex traits (e.g. blood cell phenotypes) and various disease outcomes (e.g. diabetic kidney disease development) reside within the non-coding regions of the genome [3]. Although non-coding, these regions comprise cell-type specific gene regulatory components, such as enhancers. The consortium ENCODE (ENCylcopedia Of DNA Elements) was created to identify cell type-specific gene regulatory elements including new transcript variants and transcriptional units using various human cell lines [4, 5].

The promoters of genes have regions enriched in cytosine and guanine that are referred to as CpG islands (CGI). The CGIs are 300–3000bp stretches that may be methylated on cytosines. Methylation of cytosine nucleotides can also occur outside of the CGI [6]. It is estimated that there are 27,000 CGIs in the human genome [7]. Despite having a high G+C content, the vast majority of the promoter-associated CGIs are unmethylated, as is characteristic of the promoters of constitutively expressed genes and 40 % of tissue-restricted genes [8, 9]. The lack of methylation renders the chromatin transcriptionally permissive. On the other hand, the proportion of hypermethylated CGIs is estimated at 9 – 25 % and localizes to regions distal to promoters. Hypermethylated CGIs are a hallmark of inactivated X-chromosomes, imprinted genomic regions and improperly silenced genes in neoplastic tissues. Cytosine methylation typically conforms to a transcriptionally repressed state [10] and is mediated by DNA methyl-transferases (DNMTs). With the exception of imprinted DNA segments, fertilization removes all pre-existing cytosine methylation signatures [11]. In humans, DNMT3 (A and B) mediates *de novo* methylation, while DNMT1 is engaged in the perpetuation of established methylation states during cell divisions. Elevated levels of cytosine methylation correlate with a more differentiated state. Therefore, CGIs normally unmethylated in the germline are methylated in somatic tissues.

In embryonic stem cells methylated CGI-promoters have been found on developmental genes such as *Rhox2* [12].

Epigenetic non-coding RNA modifiers

Long and short non-coding RNAs (ncRNA) are also believed to be significant in the epigenetic regulation of chromatin states [13–16]. This is best exemplified by the ncRNA, *Xist*, which is involved in random X-chromosome inactivation. It does so by recruiting Polycomb group proteins like Ezh2 which mediates methylation of lysine 27 residue on histone3 tails (H3K27me3) causing epigenetic silencing of the affected Xchromosome [16, 17].

Epigenetic Histone marks and modifiers

Chromatin organization and regulation of gene function rely on a unique combination of post-translational modifications to histone tails - the "Histone code" [18]. The "histone code" serves as a form of encrypted information about the nature and position of specific post-translational modifications within the regulatory elements in a genome. In an effort to understand the epigenetic signature of the genome as a whole (epigenomics), several consortia have been established. Among them are ENCODE (mentioned earlier), Roadmap Epigenomic Mapping Consortium (REMC), and International Human Epigenome Consortium; all conceived to provide a sweeping profile of the epigenomic landscape from several primary and cultured human cells and tissues [19–21]. Synthesis of data from these large-scale epigenome projects have provided the chromatin "fingerprints" of active, poised and repressed gene regulatory elements, such as proximal or distal promoters and enhancers [3, 22]. Access to these datasets is available through the following web links: http://www.encode-roadmap.org or http://genome.ucsc.edu/ENCODE

It is important to appreciate that the chromatin landscape, although stable between cell divisions, is liable to change in response to environmental and metabolic stresses. Chromatin modifying enzymes are reliant on co-factors and substrates that are intermediates of cellular metabolism. Changes in cell metabolism can therefore impact the chromatin modifiers which in turn alter chromatin dynamics. Intra-uterine nutrient restriction was reported to alter the cytosine methylation profile and the expression of the *Pdx1* gene of beta-pancreatic cells [21, 23]. Consequently, insulin secretion becomes impaired in these experimental models. Re-organization of the histone signature during in-vitro culture conditions has been reported by Zhu et al [21].

Histone modification patterns

Typically, H3K9me3 (tri-methylation of lysine 9 on histone 3) is a repressive mark found on heterochromatin. It renders the chromatin refractory to reprogramming during induced pluripotent stem cell (iPSCs) generation [24]. By contrast, active promoters feature tri-methylation of H3 Lysine 4 (H3K4me3) and acetylation of H3 Lysine 9 (H3K9Ac). Tri-methylation of H3 Lysine 36 (H3K36me3) is associated with the gene body of actively

transcribed genes. Active enhancers have H3K4me1 and H3K27Ac marks while "poised" enhancers bear only the H3K4me1 mark [21, 25, 26].

Chromatin modifications direct cell lineage restriction

The establishment of the metazoan body plan is complex, requiring the correct spatial and temporal specification of cellular identities [27]. In the fly, the body segments are specified by the homeotic gene cluster whose regionalized expression is epigenetically regulated by two major complexes - Polycomb (PcG, repressive) and Trithorax (trxG, activating). The Polycomb complex methylates lysines 9 and 27 on histone H3, while the Trithorax complex methylates lysines 4 on histone H3. Thus, lineage commitment and the acquisition of positional identity are driven by epigenetic modifications that regulate chromatin structure, DNA accessibility and ultimately gene expression at developmental loci. Burney et al [28] compared the epigenetic status of lineage-regulated genes in pluripotent embryonic stem cells (ESCs) as they progressed toward multi-potency (neural stem cells) and finally committed to a neural cell fate. In the pluripotent state, the promoters of lineage-specific genes carry both H3K4me3 (activating) and H3K27me3 (repressive) modifications. This "bivalent" epigenetic status is believed to keep the genes transcriptionally silent although poised for expression in response to the appropriate differentiation cues. Therefore, in the neural progenitors while both epigenetic marks are retained on the promoters of neural developmental genes they soon resolve into "monovalent" H3K4me3 status in the early, immature neurons. However, following neuronal commitment the genes specifying non-neuronal fates are repressed either by the loss of "bivalency" or the retention of the repressive mark H3K27me3 on their promoters.

Maintenance of the adult neural stem cell niche in rodents and non-human primates (in baboons) relies on the chromatin repressive mark H3K9me3 [29]. Enrichment of H3K9me3 in the germinal niche of adult brain ventricles helps maintain cellular integrity by silencing genes that promote neuronal, hepatic and immunological fates. A study by Wen et al [30] found that broad domains of H3K9 di-methylation (H3K9me2) distinguish differentiated cells from ES cells. Typically, the percentage of the genome with H3K9me2 modification increases in differentiated tissues. In mammals about 30 % of the genome carries H3K9me2 modifications. These large organized chromatin K9 modifications (LOCKs) are essential to tissue-specific gene expression and are largely lost in human cancer cell lines.

Nephric lineage epigenetic landscape

The paired domain transcription factor, Pax2, is pivotal in the epigenetic specification of the definitive kidney (metanephros) from the caudal intermediate mesoderm. Responding to inductive signals, Pax2 recruits a histone H3K4 methyltransferase complex (PTIP-MLL complex) to nephric lineage genes. Pax2 associates with this complex by binding PTIP [31–33].

Two tissue lineages both arising from the caudal intermediate mesoderm form the metanephros. One is epithelial and forms the ureteric bud (UB) and ultimately collecting duct structures, while the other is a mesenchymal precursor to all the nephrons, stroma and

parts of the vasculature (e.g., glomerular arterioles and juxtaglomerular cells). Both lineages undergo progressive fate restriction through reiterative, reciprocal signaling events. Subsets of the metanephric mesenchyme (MM) aggregate to form caps around each of the branching UB tips. The cap mesenchyme (CM) is a multipotent cell population representing progenitors of nephrons within the kidney [34, 35]. The CM cells have the ability to undergo self-renewal but remain "poised" to undergo differentiation in response to inductive Wnt signals from the UB [36]. The CM can be spatially demarcated into three sub-domains. A self-renewing population (Six2^{high}/Cited1$^+$/β-catenin^{low}) positioned immediately dorsal to the Ub tip; lateral to the Ub tip is the Six2^{high}/Cited1$^-$/BMP$^{SMAD-responsive}$ population which is primed for β-catenin-mediated differentiation and can be regarded as "poised"; further ventral to the Ub tip is Six2^{low}/Cited1$^-$/β-catenin^{high}/Wnt4$^+$ population which is committed to nephron formation (Figure 1).

Initial forays into defining the chromatin signature in the nephron lineage were done *in vitro*. Whole genome Chip-seq and Chip-qPCR was used to compare two MM cell lines [37] which are broadly representative of the self-renewing Six2^{high}/Wnt^{low} cap cells (MK3 cell line) and nephron-fate committed Six2^{low}/Wnt^{high} cells (MK4 cell line). With the onset of differentiation the MK3 cell line shows a loss of repressive histone marks, H3K9me2 and H3K27me3, and the retention of the activation mark, H3K4me3 on the promoters of nephrogenic lineage genes (e.g., *Pax2, Pax8, Lhx1, Jag1* and *Lef1*). This conforms to observations in pluripotent cells wherein genes essential for lineage commitment carry permissive histone modifications and appear poised for differentiation. Accordingly, these genes typically possess a bivalent chromatin code: broad H3K27me3 enriched domains together with several peaks of H3K4me3 positioned around the transcriptional start site. Wnt signaling is the major trigger driving the commitment and differentiation events during nephrogenesis [35]. Wnt3a could substitute for Wnt9b in inducing nephrogenic gene expression in MK3 cells. In these assays Wnt-responsive genes gain β-catenin/H3K4me3 binding and lose Ezh2/H3K27me3 at the TCF/LEF binding sites [37, 38]. A departure from the H3K4/K27 bivalent code is seen in the pancreatic lineage gene *Pdx1* in hepatoblasts [38]. *Pdx1* silencing is accomplished by H3Kac/H3K27me3 instead. By contrast, the promoters of renewal genes, *Six2, Osr1* and *Eya1* show a gain of repressive marks and the loss of the activation histone marks.

Our laboratory has also examined the spatio-temporal expression of the histone methylation marks in nephron progenitors and their progeny during mid-organogenesis [39]. Notably, the self-renewing Six2$^+$ nephron progenitors have higher levels of H3K9me2 and H3K27me3, both of which are associated with transcriptional repression of genes (Figure 1). The corresponding histone modifiers, G9a and Ezh2 show a similar distribution. Commitment of these cells to the nascent nephron fate coincides with the loss of these repressive marks. By contrast, the distribution of the activation mark, H3K4me3 does not show any spatial bias between nephron progenitors and nascent nephrons. Elevated expression of H3K9me2 was previously reported in mitotically active cells of the developing neural tube in mice [40]. This would suggest that repressive histone marks may be a signature of actively proliferating cells. Terminal epithelial differentiation of maturing nephrons is accompanied by an increase in H3K79me2/3 and its histone modifier, Dot1L. Therefore, the spatially-defined epigenetic

modifications and their modifiers guide differential gene regulation during nephrogenesis under normal physiological states [41].

Biological processes such as cell proliferation, differentiation and survival in the developing kidney can also be regulated by chromatin modifiers affecting the acetylation status of specific gene loci. This is accomplished by two opposing classes of enzymes: (1) histone acetyl transferases or HATs which add acetyl groups to histone and nonhistone proteins, and (2) histone deacetylases (HDACs; Classes I-IV) that remove these modifications. In zebrafish, HDACs act to prevent renal progenitor expansion [42]. Using pharmacological inhibitors of HDACs we identified key signaling pathways being modulated during renal development in the mouse [43, 44]. These included the Wnt/β-catenin, TGFβ/Smad and PI3K-AKT pathway components. The renal genes under HDAC modulation included *Pax2/ Pax8, Sfrp1* and *Gdnf*. Widespread cell cycle arrest and apoptosis also impairs ureteric bud branching upon inhibition of HDAC function. Congruent with this data, conditional elimination of HDAC1 and HDAC2 from the ureteric bud lineage causes renal hypoplasia owing to hyper acetylation of the tumor suppressor p53 and impaired Wnt signaling [45]. Modulation of chromatin by HDACs is also known to affect cardiovascular, oligodendrocyte, epidermal and chondrocyte development.

Clinical relevance of epigenetics

Our expanding knowledge of epigenetics can serve diagnostic and therapeutic purposes. Epigenetic characterization of a pediatric tumor, Wilm's tumor, which originates in the metanephric blastema, has been performed [46]. Using ChIP-Seq, this group mapped the promoter chromatin states of genes in this tumor relative to adult and fetal kidney. They delineated four gene sets: set 1 includes kidney-specific renewal genes (*GDNF, SIX2, EYA1, OSR1*) with broad domains of H3K4me3; set 2 comprising genes shared between Wilm's tumor and fetal kidney. These include epigenetic modulators like *JMJD2B, HDAC*s, *JARID2*, and components of the Polycomb repressive complex. These genes are believed to be relevant to lineage commitment and show H3K4me3 peaks at their promoters. Set 3 represents poised genes which are common to Wilm's tumor and fetal kidney and harbor the bivalent code H3K4me3/H3K27me3. Set 4 enlists genes that have enrichment of H3K27me3, such as repressed genes. It is surmised that the persistence of the bivalent chromatin code on the promoters of genes essential for kidney development subverts normal development in the Wilm's tumor.

Inhibitors of DNMTs (5'-azacitidine drug inhibits fibrosis in mice kidneys) or HDACs (treatment of experimentally-induced glomerulosclerosis and acute kidney injury) could have clinical relevance [47, 48]. However, owing to the pleiotropic effects of the available drugs, efforts to improve specificity of action is essential before any kind of human trials.

Acknowledgments

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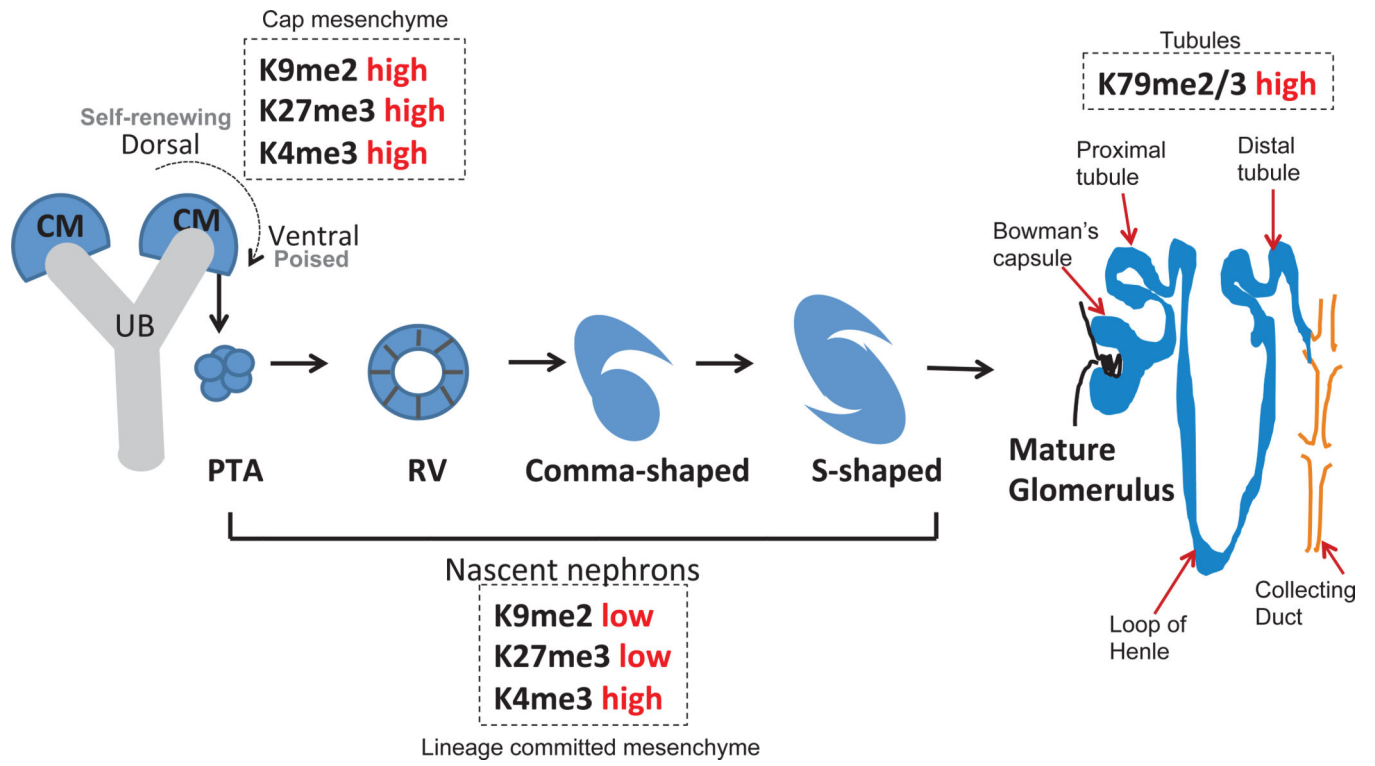
Figure 1.
Bivalent chromatin code and its role in fate determination during nephrogenesis.