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Epigenetic States of Nephron Progenitors and Epithelial Differentiation

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ABSTRACT

In mammals, formation of new nephrons ends perinatally due to consumption of mesenchymal progenitor cells. Premature depletion of progenitors due to prematurity or postnatal loss of nephrons due to injury causes chronic kidney disease and hypertension. Intensive efforts are currently invested in designing regenerative strategies to form new nephron progenitors from pluripotent cells, which upon further differentiation provide a potential source of new nephrons. To know if reprogramed renal cells can maintain their identity and fate requires knowledge of the epigenetic states of native nephron progenitors and their progeny. In this article, we summarize current knowledge and gaps in the epigenomic landscape of the developing kidney. We now know that Pax2/PTIP/H3K4 methyltransferase activity provides the initial epigenetic specification signal to the metanephric mesenchyme. During nephrogenesis, the cap mesenchyme housing nephron progenitors is enriched in bivalent chromatin marks; as tubulogenesis proceeds, the tubular epithelium acquires H3K79me2. The latter mark is uniquely induced during epithelial differentiation. Analysis of histone landscapes in clonal metanephric mesenchyme cell lines and in Wilms tumor and normal fetal kidney has revealed that promoters of poised nephrogenesis genes carry bivalent histone signatures in progenitors. Differentiation or stimulation of Wnt signaling promotes resolution of bivalency; this does not occur in Wilms tumor cells consistent with their developmental arrest. The use of small cell number ChIP-Seq should facilitate the characterization of the chromatin landscape of the metanephric mesenchyme and various nephron compartments during nephrogenesis. Only then we will know if stem and somatic cell reprogramming into kidney progenitors recapitulates normal development. J. Cell. Biochem. 116: 893–902, 2015.

KEY WORDS: KIDNEY DEVELOPMENT; EPIGENETICS; CHROMATIN SIGNATURE; NEPHROGENESIS; NEPHRON PROGENITORS

Chromatin is a key regulator of all DNA-templated cellular processes. From gene transcription to DNA replication and repair, numerous vital activities are tightly controlled by chromatin structure, which tightly packs nearly 2-meter long DNA molecule into nucleus of every human cell. The basic building blocks of chromatin are nucleosomes which are composed of DNA molecule wrapped around histone protein dimers (H2A, H2B, H3 and H4). Nucleosomes organization is neither random nor uniform across the genome. Genomic information is partitioned into tightly packaged "closed" compartments called heterochromatin and actively regulated "open" chromatin regions called euchromatin [Zhou et al., 2011]. Notably, throughout developmental and cellular differentiation, chromatin structure is dynamically regulated to allow expression of lineage specific genes from the same genome. Thus, the information that regulates lineage or cell type specific gene expression program is not genetic information. Broadly speaking, nearly 200 different cell types in our body carry the same genetic information and yet they are functionally and phenotypically different. The information that provides memory for lineage specific gene expression program is epi-genetic (above-genetic) information.

EPIGENETIC INFORMATION

The word epigenetic is contentious and still debatable but epigenetic can be broadly defined as the mechanism that mediates heritable changes in gene expression without changes in genetic information [Berger et al., 2009]. By this definition, it can be considered that

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normal development is an epigenetic process [Shen and Orkin, 2009]. Epigenetic information is coded in the chromatin structure in the form of chemical modifications to DNA and histone proteins as well as regulatory non-coding RNA molecules. In this review, we will briefly touch upon the role of DNA methylation and non-coding RNAs and focus on the histone modifications.

DNA METHYLATION

DNA methylation is a biochemical process where a "methyl" group is covalently attached to 5th carbon of "cytosine" (C) nucletodies (5C-methylation). Although it was initially thought that only cytosines in cytosine-guanosine dinucleotide pairs (CpG) could be modified, later studies demonstrated that other non-CpG cytosine residues are also methylated [Lister et al., 2009; Lister et al., 2013]. DNA methylation is historically associated with transcriptional repression [Wu and Zhang, 2014]. However, it should be noted that in addition to 5C-methylation, the cytosine residues are also hydroxymethylated, formylated and carboxylated [Kriaucionis and Heintz, 2009; Tahiliani et al., 2009]. Although the functional roles of these modifications are not fully understood, they are considered to be the intermediate modifications of DNA demethylation process mediated by ten eleven translocation (TET) DNA demethylase enzymes [Kohli and Zhang, 2013]. The methylation process is mediated by DNA methyl-transferases (DNMTs). In humans, DNMT3A and DNMT3B are involved in de novo DNA methylation process where as DNMT1 is implicated in the maintenance of DNA methylation [Wu and Zhang, 2014].

NON-CODING RNAS AS EPIGENETIC MEDIATORS

Chromatin regulatory function of non-coding RNAs is being increasingly recognized. Human genome encodes many thousands of short and long non-coding (nc) RNAs; however, the molecular function of the vast majority of these is yet to be discovered [Cheng et al., 2005; Mercer et al., 2009; Derrien et al., 2012]. Seminal work on several long ncRNAs has shown their vital functions in epigenetic gene regulation and global chromatin organization [Rinn et al., 2007; Lee and Bartolomei, 2013]. One of the best example is *Xist* ncRNA, an essential player in X-Chromosome inactivation in mammals [Lee and Bartolomei, 2013], where one of the X chromosomes is randomly silenced by *Xist* mediated polycomb group proteins. Xist mediates orchestrated recruitment of key components of polycomb group proteins such as EZH2, which leads to deposition of repressive H3K27me3 histone modification and epigenetic silencing of X-chromosome [Zhao et al., 2008].

HISTONE MODIFICATIONS

Histones are major protein components of nucleosomes. Post-translational modifications on histone tails contribute to the majority of epigenetic information. High throughput mass spectrometry analysis shows that there are over hundred different kinds of modifications in the histone tails [Tan et al., 2011]. Among these modifications, the most widely studied forms are methylation, acetylation and phosphorylation. Importantly, the specific location of the modification is as important as the modification itself. For example, tri-methylation of lysine 4 position of histone H3 tail (H3K4me3) is associated with active promoters, whereas H3 Lysine 27 tri-methylation (H3K27me3) is associated with Polycomb mediated gene repression [Zhou et al., 2011] (Fig. 1). It has been postulated that a combination of different histone modifications form a "Histone Code" that dictates a specific structure and function in the chromatin [Strahl and Allis, 2000]. Histone modifications are believed to affect chromatin function and gene expression in two ways. Firstly, they may physically alter the affinity of DNA and histone proteins thus making DNA less or more accessible for

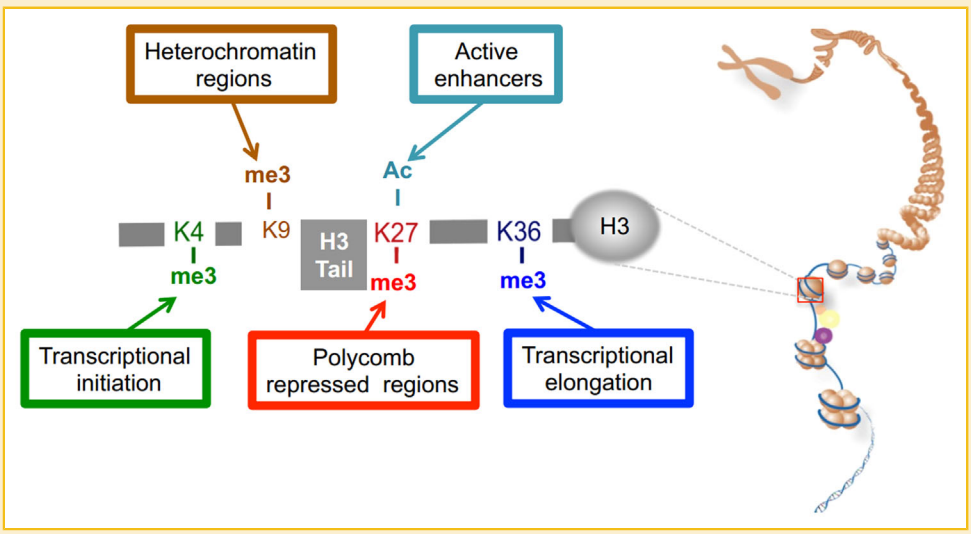

Fig. 1. Major post-translational histone modifications on Histone H3 tail are schematized. Genomic regions or activity that are associated with each modification is indicated in writings. For example, tri-methylation H3 Lysine 4 position (H3K4me3) is associated with transcriptional activation, whereas tri-methylation of Lysine 27 position (H3 K27me3) is associated with Polycomb mediation repression.

transcription. Secondly, they may act as binding sites for other chromatin modifiers and remodeling complexes that regulate local chromatin function. Considering the vast number of modifications to DNA and histones and their potential combinations, it becomes clear that chromatin encodes significantly larger amount of information compared to the four-letter genetic code.

MAPPING WHOLE GENOME LEVEL CHROMATIN MODIFICATIONS

The challenge is to figure out the biological meaning of vast number of chromatin modifications. Although biochemical and genetic studies taught us the importance of various modifications, recent whole genome mapping studies significantly advanced our understanding of epigenetic marks in the context of gene regulation and genome organization. Several large-scale consortia such as the Encyclopedia of DNA Elements (ENCODE) [Consortium et al., 2012] and Roadmap Epigenomic Mapping Consortium (REMC) [Bernstein et al., 2010] projects mapped various chromatin modifications in wide range of primary and cultured human cells. The goal is to acquire and integrate many reference epigenomic maps in order to gain insight into temporal, spatial and cell type specific genome regulation and organization [Adli et al., 2010]. These whole genome level chromatin-mapping efforts revealed unprecedented information regarding specific epigenomic features at regulatory genomic regions such as gene proximal promoters and distal enhancers in the genome [Bernstein et al., 2010; Consortium et al., 2012; Zhu et al., 2013] (Fig. 2).

EPIGENETICS VS EPIGENOMICS

It should be noted that the words "epigenetics" and "epigenomics" are sometimes used interchangeably but they have different meanings. Epigenetics describes DNA-sequence independent mechanism of gene expression and chromatin function. On the other hand, Epigenomics is full description of various chromatin modifications at the whole genome level. Certain chromatin marks such as Lysine 36 tri-methylation of Histone H3 (H3K36me3) that is observed in the gene body of actively transcribed genes is an important epigenomic features. However, the epigenetic function of this mark is yet to be understood.

EPIGENOMIC TOOLS FOR HISTONE MODIFICATIONS

Whole genome level mapping is key to identifying potential regulatory functions of a novel mark or to figure out the cell type specific whole-genome distribution patterns. Current gold standard technology of mapping histone modifications and protein-DNA interactions is chromatin immunoprecipitation and high throughput sequencing (ChIP-Seq) technology [Landt et al., 2012]. ChIP-Seq enables high-resolution mapping of transcription factor-DNA binding sites and genomic regions marked by a specific histone modification. ChIP-Seq has many advantages compared to previous array based chip technologies; it is truly whole genome and provides single nucleotide resolution. However, the inherent limitations of ChIP experiments are the requirement for large number cells (10–50 millions) and the necessity of significant amount of DNA for current DNA sequencing platforms (5–10 ng of ChIP DNA). These challenges make ChIP-Seq technology unavailable for various biologically important but limitedly available samples. To overcome these challenges, a number of protocols have been devised. We previously developed a Nano-ChIP-seq technology that enables epigenomic profiling of histone modifications in as few as 10,000 cells [Adli et al., 2010; Adli and Bernstein, 2011]. Nano-ChIP-Seq technology enabled epigenomic profiling of in vivo isolated hematopoietic stem and progenitor cells for the first time [Adli et al., 2010]. In addition to Nano-ChIP-Seq technology, alternative library preparation protocol called LINDA amplification has been proposed to profile chromatin in as few as 5,000 cells [Shankaranarayanan et al., 2011]. Notably, more recently, additional protocols have been published that enables

Chromatin state	H3 K4me1	H3 K4me3	H3 K9ac	H3 K9me3	H3 K27ac	H3 K27me3	H3 K36me3
Active Promoter		✔	✔		✔		
Inactive/poised promoter		✔				✔	
Active enhancer	✔				✔		
Inactive/poised enhancer	✔						
Transcriptional elongation							✔
Polycomb repressed						✔	
Stable heterochromatin				✔			

Fig. 2. Chromatin states of regulatory genomic regions are tabulated. Whole-genome mapping of histone modifications reveals specific epigenomic features at regulatory genomic regions. Integrative analysis of these marks in a given cell type allows identification cell type specific chromatin states at regulatory genomic regions such as promoters and enhancers.

profiling in even fewer cell numbers. The iChIP protocol claims to allow ChIP-Seq analysis in as few as 500 cells by indexing the fragmented chromatin before immunoprecipitation [Lara-Astiaso et al., 2014].

All these ChIP-Seq protocols are antibody based, thus the quality of the data is directly linked to the quality of the antibody utilized. Notably, there are antibody independent chromatin profiling tools such as DNAse-Seq technology that allows mapping open chromatin regions in the genome. Since a specific antibody is not utilized, the method allows identification of potentially all DNAse 1 accessible chromatin sites, that is, open chromatin regions in the genome regardless of the chromatin state or the factors bound there [Thurman et al., 2012]. Like ChIP-Seq experiments, DNAse-Seq experiments also require large number of cells, typically 30–50 millions of cells. Importantly, a recently published protocol maps whole genome level open chromatin regions in the genome by utilizing a transposon. This transposase-accessible chromatin using sequencing (ATAC-Seq) method results in open chromatin maps that are highly comparable to DNAse-Seq profiles. The protocol is much simpler and more significantly it can be used to map open chromatin sites in as few as 500 cells, which is 4–5 order of magnitude less cells compared to DNAse-Seq [Buenrostro et al., 2013].

UNDERSTANDING GENOME THROUGH EPIGENOME

Large-scale epigenome projects applied various technologies to acquire epigenomic maps of multiple cell types. Integrative analysis of these epigenomic maps revealed unprecedented details of cell type specific chromatin landscapes and genome organization [Barski et al., 2007; Ernst et al., 2011]. These maps identified specific epigenomic features associated with a wide range of regulatory elements in the genome (Fig. 2). Detailed chromatin state maps of active promoters, repressed promoters, poised promoters, expressed gene body regions, active and poised distal enhancer regions are all available for various cell types [Ernst et al., 2011; Consortium et al., 2012; Zhu et al., 2013] (Fig. 2). All these maps are publically available to download or browse through user-friendly websites such as http://www.encode-roadmap.org or http://genome.ucsc.edu/ENCODE.

In addition to gene specific chromatin states, epigenomic maps also revealed various large-scale chromatin state transitions associated with environmental and developmental cues. Integrative analysis of multiple histone modification maps and histone variant across more than 30 different human tissues revealed large-scale chromatin re-organizations during in vitro culturing conditions [Zhu et al., 2013]. Mega-base size H3K9me3 marked heterochromatin domains arise during in vitro culturing conditions [Zhu et al., 2013]. Importantly, these domains may be refractory to proper reprogramming during epigenetic reprogramming into induced pluripotent stem cells [Lister et al., 2011; Onder et al., 2012]. Inline with these studies, it has recently been shown that genomic regions marked by H3K9me3 methylation impede embryonic development during somatic cell nuclear transfer [Matoba et al., 2014]. These results suggest that H3K9me3 domains form stable heterochromatin domains in the genome of in vitro cultured and terminally differentiated cells. In contrast to repressive chromatin modifications, biochemical studies followed by extensive genome-wide chromatin mapping showed numerous histone modifications marks associated with actively regulated genomic regions. Among these chromatin marks, several of them are well studied because they provide key insight into epigenomic features at active regulatory genomic regions. Integrative analysis of chromatin maps yielded unique chromatin states decorated by combination of chromatin marks. For example, major marks for active promoter regions are H3 lysine 4 tri-methyaliton (H3K4me3) and H3 Lysine 9 acetylation (H3K9Ac). Another important chromatin mark for active genes is tri-methylaiton of histone H3 Lysine 36 position (H3K36me3). This mark is associated with transcriptional elongation and nicely decorates the gene body of actively transcribed genes. Notably, this marks is also implicated in splicing [Luco et al., 2010].

In contrast to proximal regulatory elements, distal regulatory regions such as enhancers are marked by another distinct chromatin marks. For example, active enhancer regions are marked with H3K27ac and H3K4me1. Although it is yet to be fully understood, it is considered that poised enhancers are marked by H3K4me1 mark alone [Heintzman et al., 2009; Rada-Iglesias et al., 2011; Zhu et al., 2013]. In addition to activating histone modifications, actively regulated genomic regions such as promoters and enhancers are DNAse1 accessible chromatin sites. Therefore, these sites feature significant DNAse1 hypersensitivity in DNAse-Seq experiments [Thurman et al., 2012] (Fig. 2).

IDENTIFYING DISEASE ASSOCIATED ABERRANT EPIGENOMIC FEATURES

Importantly, mutations in histone modifiers (enzymes which add or remove various histone marks) are consistently found in many diseases including cancer [Berdasco and Esteller, 2010; Brower, 2011; Shen and Laird, 2013]. ENCOCE type large-scale projects revealed large number of reference epigenomic maps for wide range of tissues and cell types. Similar epigenome mapping strategies can be utilized to map chromatin features in disease states and these maps can be comparatively analyzed with the normal reference epigenomes to identify disease-specific aberrant chromatin features. Such analyses may reveal unprecedented insight into disease state and identify potential therapeutic targets. For example, through epigenomic profiling, we recently discovered that ASXL1 mutations leads to cellular transformation in myeloid malignancies by causing loss of repressive H3K27me3 mark at hematopoietic stem cell genes such as HOXA cluster [Abdel-Wahab et al., 2012]. Because epigenetic information is reversible, discovering disease-associated specific aberrant epigenomic features holds promise for therapeutic interventions. Small molecule epigenetic inhibitors are seen as a potentially viable arsenal for various diseases and a number of such inhibitors are already FDA approved or are in clinical or preclinical trials [Dawson et al., 2012].

EPIGENETIC SPECIFICATION OF THE NEPHRIC LINEAGE

The metanephric kidney originates from the intermediate mesoderm (IM) via reciprocal interactions between two primordial cell lineages, the metanephric mesenchyme (MM) and the ureteric bud (UB), an epithelial outgrowth of the nephric duct (reviewed in [Costantini and Kopan, 2010; Dressler, 2011]). The Paired Domain transcription

factor, Pax2, is required for patterning and elongation of the nephric duct and for survival of the MM [Torres et al., 1995].

In addition to this function, the Pax2 protein is one of the earliest epigenetic regulators of the metanephric kidney. Pax2 associates with a histone methyltransferase complex containing the Trithorax homologues ALR/Mll2 and Mll3 through interactions with the adaptor protein, PTIP [Patel et al., 2007]. PTIP is part of the Trithorax histone H3K4 methyltransferase complex [Cho et al., 2007]. The interaction of Pax2 with PTIP is believed to promote H3K4 methylation at kidney-specific loci in response to inductive signals providing locus and tissue specificity for genetic and epigenetic signals to restrict the developmental potential of the IM to the renal lineage [Patel et al., 2007] (Fig. 3A). Although PTIP mutants die early in embryogenesis, and PTIP is important for podocyte, collecting duct cell and cardiomyocyte function [Dressler and Patel, 2015],

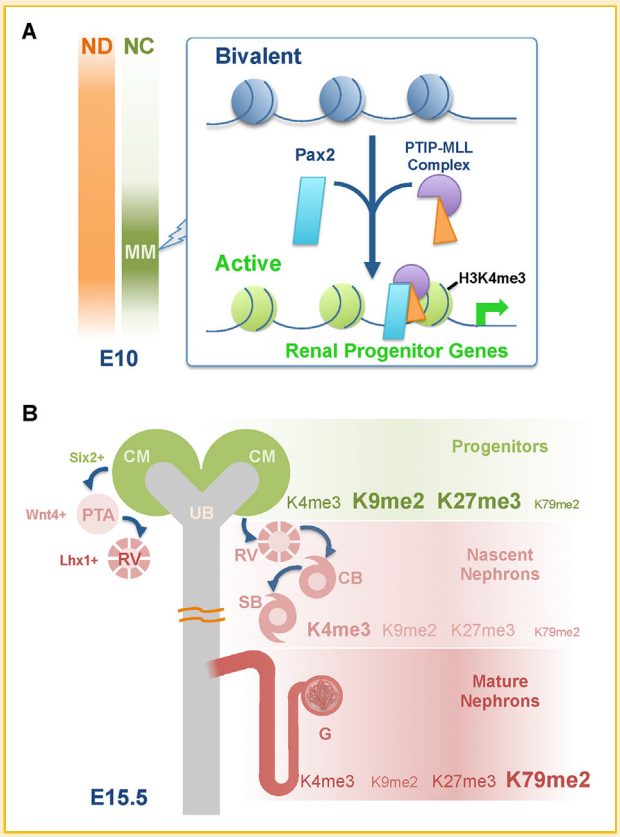

Fig. 3. (A) Epigenetic specification of the metanephric mesenchyme in the intermediate mesoderm prior to the onset of metanephric development (embryonic day 10). Regulatory elements of the earliest renal developmental genes carry bivalent histone marks. The transcription factor Pax2 specifies the nephric lineage by recruiting an activating histone methyltransferase complex (PTIP/MLL) thus activating nephric lineage genes [Patel and Dressler, 2013; Dressler and Patel, 2014]. (B) The histone H3 signature of Six2 stem cells and progeny as assessed by section immunofluorescence at embryonic day 15.5. Repressive histone marks are abundant in the cap mesenchyme, whereas H3K79me2 is more abundant in differentiated tubules and podocytes [McLaughlin et al., 2014]. ND, nephric duct; NC: nephrogenic cord; PTA: pre-tubular aggregate; RV: renal vesicle; CB: C-shaped body; SB: S-shaped body; G: glomerulus; UB: ureteric bud.

the precise role of PTIP in early kidney development remains to be defined. Technical advances in chromatin biology, such as iChIP and ATAC-Seq (described above), which can overcome the cell number barrier, should allow the identification Pax2/PTIP-target genes involved in the specification of the caudal IM. This information is critically needed to fully understand the epigenetic basis of nephric lineage specification.

NEPHRON PROGENITORS ARE PRIMED FOR DIFFERENTIATION

Following the invasion of the MM by the UB and initiation of branching morphogenesis, a group of $Six2^{+}$ MM cells condenses around the UB branch tips to form a crescent-shape progenitor niche (also called the capping mesenchyme). Lineage fate analysis of Six2 cells revealed that they give rise to all cell types of the nephron from the glomerulus to the connecting tubule [Kobayashi et al., 2008; Park et al., 2012], whereas the collecting ducts are derived from the UB lineage. Nephron progenitors express "stemness" genes such as *Cited1*, *Six2*, *Eya1*, *Sall1*, and *Osr1*, and have self-renewing properties, but are also poised to undergo mesenchyme-to-epithelium transition (MET) in response to inductive Wnt signaling emanating from the adjacent UB [Carroll et al., 2005]. In this regard, the transcription factor Six2 interacts and cooperates with Lef/Tcf factors and β-catenin to initiate expression of Wnt-dependent nephrogenic genes, which fall under two major classes: Class I genes, such as *Wnt4*, *Lhx1*, *Jagged1*, *Pax8*, and *Fgf8*, are expressed in nascent nephrons (e.g., renal vesicle) and require low Six2 and high β-catenin levels for induction, whereas Class 2 genes (e.g., *Tafa5*, *Eya1*) are expressed in the cap mesenchyme and respond to conditions of high Six2 and low β-catenin levels [Karner et al., 2011; Park et al., 2012]. Targeted deletion of the stemness factor, Six2, in nephron progenitors de-represses the differentiation program triggering premature nephrogenesis, eventually resulting in nephron progenitor depletion and reduced nephron endowment [Self et al., 2006]. Interestingly, overexpression of Notch or inactivation of Fgf signaling in the cap mesenchyme also triggers premature differentiation of nephron progenitors [Boyle et al., 2011; Barak et al., 2012], suggesting that Six2 stem cells are "primed" for differentiation, and raises the question of whether chromatin-based mechanisms balance the cell fate decisions between renewal and differentiation in this multipotent population.

THE HISTONE MODIFICATIONS LANDSCAPE OF THE NEPHROGENIC NICHE

$Six2^{+}$ nephron progenitors, which form a crescent shaped niche around the UB branch tip, are a heterogenous cell population composed of at least three sub-populations: a) self- renewing stem cells ($Six2^{high}$/$Cited1^{+}$/β-$catenin^{low}$/$BMP^{MAPK\text{-}responsive}$). This cell population responds to Wnt signaling by proliferation; b) a transit $Six2^{high}$/$Cited1^{-}$/$Wnt4^{-}$/$BMP^{Smad\text{-}responsive}$ population which is highly responsive to β-catenin-mediated differentiation; and c) $Six2^{low}$/$Cited1^{-}$/β-$catenin^{high}$/$Wnt4^{+}$ cells undergoing MET (pre-tubular aggregates). Nascent nephrons undergo lumen formation, elongation, polarization, segmentation, and functional acquisition. Epigenetic mechanisms have been implicated in cell fate decisions of cap mesenchyme cells [Surendran and Kopan, 2011], but little is known about the global epigenetic states of this lineage-committed

progenitor population. To shed some lights into the chromatin landscape of nephron progenitors and progeny, we examined the spatiotemporal expression patterns of H3K methyl marks and respective histone methyltransferases (HMT) in E15.5 mouse kidneys undergoing active UB branching and nephrogenesis [McLaughlin et al., 2014]. The results revealed that H3K9me2 and H3K27me3, chromatin marks associated with transcriptional repression, are more enriched in $Six2^{+}$ progenitors than nascent nephrons. This correlates with differential expression of the respective histone modifiers, G9a and Ezh2, in these two compartments [McLaughlin et al., 2014]. In this regard, Biron et al. [Biron et al., 2004] reported that H3K9me2 is highly abundant in mitotic cells lining the medial edges of the neural tube in E9.5 embryos. Together, these data suggest that repressive histone marks defines embryonic regions with high proliferative activity.

Compared to cap mesenchyme cells, nascent nephrons retain high H3K4me3 but show downregulation of H3K9/K27me3, whereas maturing epithelial tubules acquire high levels of H3K79me2. Temporally, while the global levels of most histone H3 marks remain stable during nephrogenesis, H3K79me2/3 (and the responsible HMT, Dot1l) showed remarkable upregulation during terminal epithelial differentiation [McLaughlin et al., 2014]. Collectively, these data indicate that nephron progenitors and differentiating daughter cells exhibit common yet distinct histone signatures under normal physiological states, and that nephrogenesis is accompanied by dynamic changes in histone modifications characterized by retention, gain or depletion of histone marks (Fig. 3B). We propose that these combinatorial signatures (epigenetic states) may be important in defining the fate of stage- specific nephron differentiation. Ultimately, the functional significance of these spatiotemporal changes will be revealed by stage-specific ablation of HMTs in mice.

GENOME-WIDE CHIP-SEQ ANALYSIS OF METANEPHRIC MESENCHYME CELL (MM) LINES

Bivalent Chromatin in "poised" genes of nephron progenitors. Defining the epigenomic landscape of nephron progenitors poses a formidable challenge given the heterogeneity of the Six2 progenitor population and the inherent difficulty in isolating sufficient numbers of pure self-renewing and differentiating cells from the cap mesenchyme. We therefore took advantage of two mouse clonal MM cell lines representing the mesenchyme ($Six2^{high}/Wnt4^{low}$) and epithelializing ($Six2^{low}/Wnt4^{high}$) populations [McLaughlin et al., 2013]. Whole genome ChIP-Seq and targeted ChIP-qPCR revealed that promoters of silent nephrogenic genes (e.g., *Pax2*,*Pax8*, *Lef1*, *Jag1*, and *Lhx1*) undergo loss of the repressive histone marks, H3K27me3 and H3K9me2, and/or gain of the active mark, H3K4me3, during differentiation. In contrast, chromatin of renewal genes (e.g., *Six2*, *Osr1*) is depleted of H3K4me3 with either retention or gain of repressive histone marks (Fig. 4). Thus, our genome-wide analysis has revealed features previously seen in pluripotent cells, in which critical genes involved in differentiation (i.e., nephrogenesis genes), despite remaining silent, exhibit a permissive chromatin structure that renders them sensitive to differentiation signals. This permissive chromatin environment is characterized by the presence of large H3K27me3 domains harboring peaks of H3K4me3 around the transcriptional start site. Our results are also in line with data comparing the epigenetic landscapes of endoderm cells and their more differentiated liver and pancreas progeny, demonstrating that chromatin of silent genes is pre-patterned with distinct histone marks and chromatin modifying enzymes [Xu et al., 2011]. In this study, Xu et al. [Xu et al., 2011] employed fluorescent-activated cell sorting (FACS) to isolate mouse ventral foregut endoderm just prior to the induction of hepatic and pancreatic fates and assessed their epigenetic landscapes using a low-cell number ChIP protocol. The results revealed that the silent liver and pancreatic regulatory elements exhibit distinct chromatin states; differentiation of foregut endoderm into hepatoblasts was associated with a significant increase in H3K9acK14ac in liver-specific (e.g., Albumin, AFP) but not pancreatic genes. H3K27me3 and its enzyme, Ezh2, were enriched at the regulatory elements of the pancreas-specific gene Pdx1 during hepatic fate differentiation. Interestingly, conditional deletion of Ezh2 in foregut endoderm cells expanded the PDX1+ ventral pancreatic domain at the expense of the liver domain. Accordingly, this study demonstrates that Ezh2 indirectly promotes the liver fate by restraining ventral pancreatic specification during endoderm differentiation. An intriguing finding of this study was that the regulatory elements in the silent Pdx1 gene in sorted hepatoblasts are occupied by a novel bivalent H3Ac/H3K27me3 mark rather than the canonical H3K4/K27 mark characteristic of pluripotent cells [Xu et al., 2011]. This finding requires confirmation in other native cell lineages.

Wnt signaling promotes an active chromatin signature in nephrogenic genes. Wnt signaling is the driving force for nephron progenitor cell differentiation *in vivo* [Park et al., 2012]. We examined the effect of exogenous activation of canonical Wnt signaling on the histone signature of class I nephrogenic genes (e.g., *C1qdc2*, *Etv5*, *Hnf1b*, *Lhx1*, *Pax8*, and *Sim1*). ChIP-PCR revealed that Wnt3a-stimulated expression of nephrogenic genes is preceded by augmented promoter co-occupancy of β-catenin/H3K4me3 and a reciprocal depletion of Ezh2/H3K27me3 around the Tcf/lef-binding sites [McLaughlin et al., 2013]. These data suggest that the critical biochemical and transcriptional pathways of MET are closely linked to the epigenetic machinery (Fig. 5). In the future, it will be important to decipher the epigenetic codes of modular enhancers in progenitor and nephrogenic genes utilizing native freshly isolated mouse and human Six2 cells.

LESSONS LEARNED FROM WILM'S TUMOR AS COMPARED TO NORMAL FETAL TISSUE

Wilms tumor is a pediatric malignancy that arises from the metanephric precursors or blastema cells. Previous analyses indicated that the tumor cells are arrested in their differentiation and tend to aberrantly express nephron progenitor markers (e.g., Six2) and high β-catenin levels [Koesters et al., 2003; Rivera et al., 2007]. Using ChIP-Seq, Aiden et al. [Aiden et al., 2010] generated genome-wide chromatin maps for human WTX mutant Wilm's tumor, normal adult kidney and fetal kidney. Four gene sets were defined based on promoter chromatin states in Wilm's tumor. Set1 is enriched in kidney development genes required for stem cell renewal and survival, for example, GDNF, SIX2, EYA1, and OSR1 with active chromatin (broad peaks of H3K4me3). This suggests

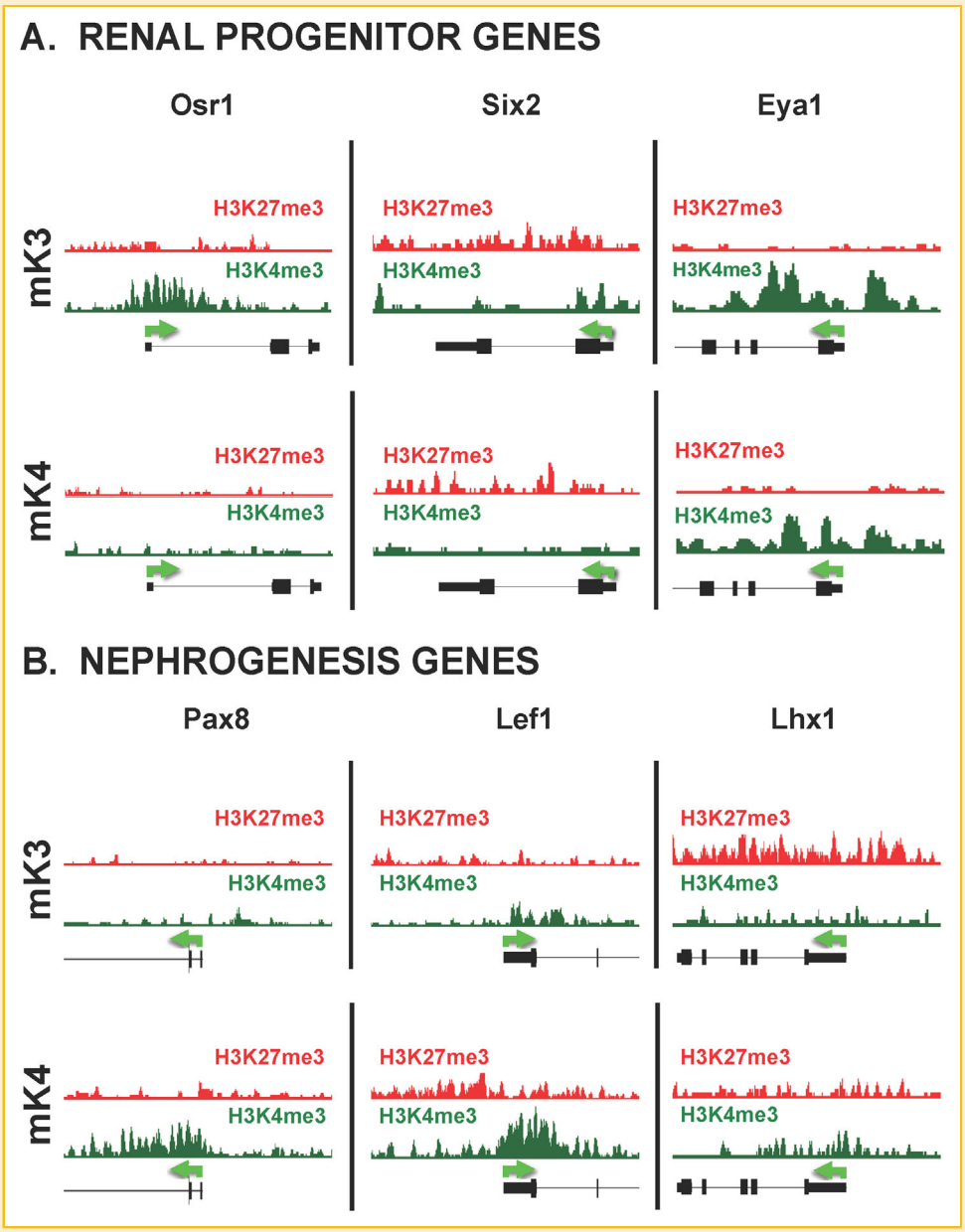

Fig. 4. ChIP-Seq tracks of renal progenitor genes (A) and nephrogenesis genes (B) in mouse clonal undifferentiated mK3 (Six2^{high}; Wnt4^{low}) and differentiating mK4 (Six2^{low}; Wnt4^{high}) metanephric mesenchyme-like cells. Repression of renal progenitor genes is generally achieved by loss of activating histone marks (H3K4me3) in the proximal promoters. In the case of Six2, gain of H3K9me2 also occurs (not shown). In comparison, nephrogenesis genes undergo a gain of H3K4me3, depletion of H3K27me3 or both during differentiation.

that Wilms tumor cells may be driven by a transcriptional pathway analogous to nephron progenitors. Set 2 contains genes with active chromatin (H3K4me3 peaks) shared in tumor cells and fetal kidney but not adult kidney. This set is enriched in epigenetic regulators such as JMJD2B, HDACs, FBXL10 (KDM2b), JARID2, and CBX2 comprising components of the Polycomb repressive complex 1 and 2, which play key roles in cell lineage commitment and maintenance during embryogenesis. Set 3 contains genes with bivalent chromatin (H3K4me3/K27me3), which correlate with silent yet poised genes. These genes lack H3K36me3 in their coding regions and are enriched in Wilms tumor and fetal kidney but not adult kidney. Since bivalent promoters in developmental genes tend to resolve during differentiation, the persistence of bivalent domains in Wilms tumor cells (e.g., *LHX1* and *FGF8*) strongly suggests that Wilms tumor cells have arrested during normal differentiation (i.e., a stalled renal differentiation program). Moreover, this raises the question that epigenetic therapy (e.g., activators of histone demethylases or HMT/HDAC inhibitors may be a useful therapeutic adjunct

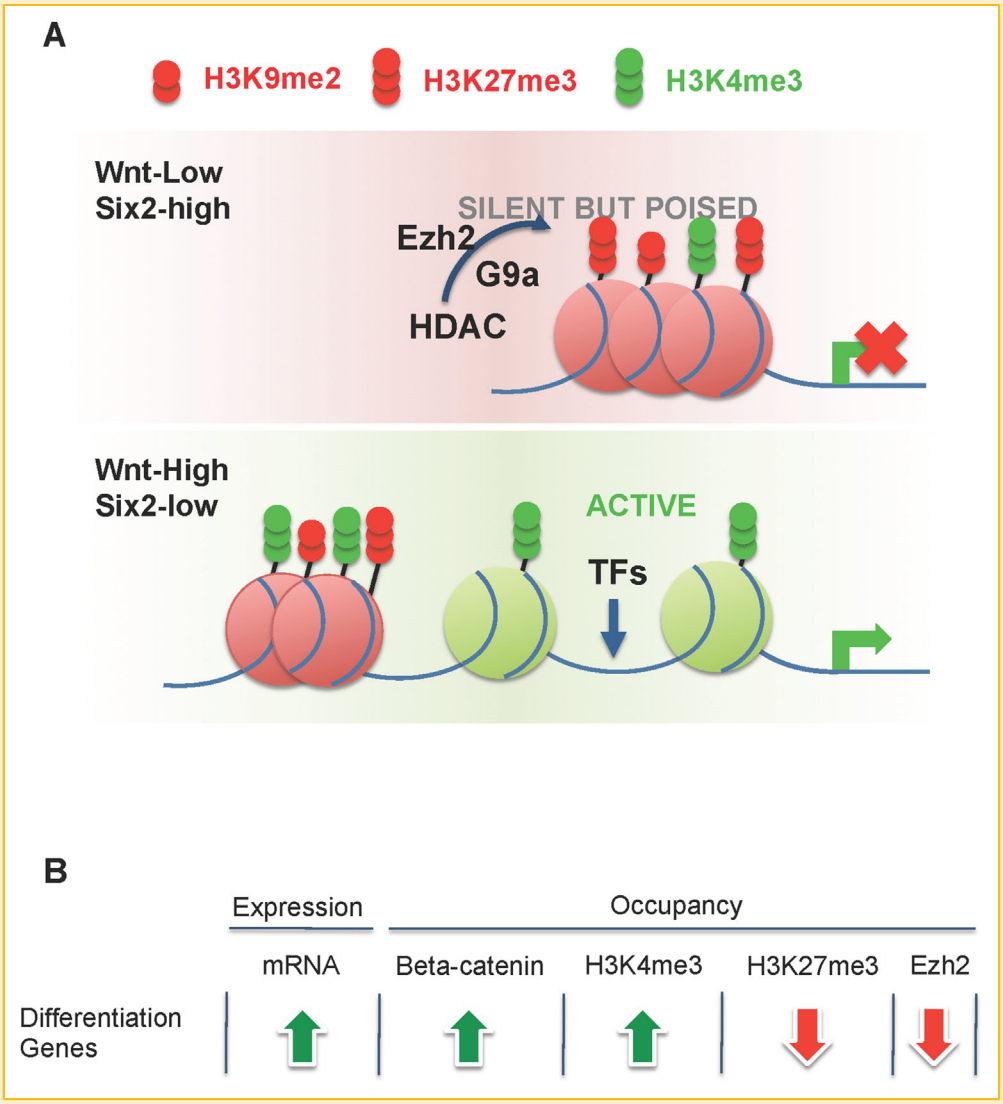

Fig. 5. (A) Working model for the epigenetic regulation of nephron progenitor cell differentiation. Six2^{high} stem cells express high levels of HDAC1/2, Ezh2, and G9a. Histone deacetylation and methylation on H3K9 and K27 of nephrogenic genes keeps them silent yet poised for differentiation (paused PolII). Downregulation of Six2 and upregulation of β- catenin, in response to Wnt signaling, promotes the remodeling of local chromatin into an accessible state allowing the displacement of the repressive epigenetic machinery and the binding of the transcription factor–active PolII complexes. (B) In response to Wnt signaling, nephrogenesis genes are induced; this is accompanied by enhanced promoter occupancy with β-catenin, H3K4me3 and reciprocal depletion of H3K27me3/Ezh2 [McLaughlin et al., 2013].

in Wilms tumor); and Set 4 represents genes enriched in H3K27me3 (mostly repressed developmental and tissue-non-specific genes) [Aiden et al., 2010]. Interestingly, Sets 1–4 are well represented in the ChIP-Seq study performed in mouse clonal MM cell lines [McLaughlin et al., 2013].

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