THE EPIGENETIC HISTONE CODE OF THE DEVELOPING NEPHRON.

Nathan McLaughlin, Fenglin Wang, Zubaida Saifudeen, Susana Dipp, and Samir S. El-Dahr.

Dept. of Ped., Tulane SOM, New Orleans, LA.
Epigenetic mechanisms are known to cooperate with transcription factors in cell fate determination. Mutations in epigenetic regulators have a profound impact on embryonic development and organogenesis. Cap mesenchyme Six2/Cited1+ cells are a group of self-renewing progenitor cells which respond to Wnt-inductive signaling by differentiating into the Lhx1+ renal vesicle, which in turn gives rise to the nephron. Little if anything is known regarding the epigenetic regulation of nephrogenesis. We hypothesize that renal progenitor cells exhibit a bivalent histone methylation mark characteristic of “stem or pluripotent” cells in the embryo. We further postulate that successive stages of nephrogenesis are marked by distinct cassettes of combinatorial epigenetic modifications (signatures).

Embryonic kidney sections (E15.5) were probed with antibodies against histone modifications or modifying enzymes and markers of differentiation. Z-plane images (>15) were acquired, deconvolved and co-localization determined.

Six2/Cited+ cells exhibited a wide range of epigenetic modifications including activating (H3K4/36me3, H3R17me2, H4R3me2) and repressive (H3K9/27me3) marks. During transition to Lhx1+ cells, there was a significant decline in both lysine and arginine methylation (H3K9me2, H3K9/27/36me3, H3R17me2, H4R3me2); in contrast, H3K4me3 and H3K79me3 were upregulated. Next, the responsible lysine methyltransferases (KMT) and demethylases (KDM) were examined. Specifically, Ash2/LSD1 (H3K4 KMT/KDM), Suvh39 (H3K9 KMT), and Dot1 (H3K79 KMT) were examined. Ash2l/H3K4me3 were expressed in Pax2+ epithelial nephron progenitors, whereas Lsd1 was most pronounced in the mesenchyme. Suvh39/H3K9me3 were present in the undifferentiated cortical mesenchyme. H3K79me3/Dot1 positive cells were enriched in the more mature collecting system.

Six2+ renal progenitor cells express both activator and repressor marks, consistent with the “bivalent mark” observed in other progenitor cell populations. Differentiation is accompanied by loss of repressive marks (e.g., H4R3me2, H3K9me3) as well as the gain and retention of activating marks (e.g., H3K79me3, H3K4me3). Importantly, the histone modifying enzymes investigated demonstrate a more compartmentalized expression, suggesting that epigenetic regulation is integral to nephrogenesis. These data provide the first description of the epigenetic signature of the developing nephron.