Title

Dynamics and heterogeneity of renal lymphatic vessel development

Authors

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Abstract

Renal lymphatic vessels have roles in fluid homeostasis and immunomodulation, and are thought to be key players in chronic kidney disease and transplant rejection. However, there is a paucity of information regarding how the lymphatic system of the kidney develops. Historically, identifying lymphatics has been challenging and requires multiple markers together with appropriate imaging techniques. We have overcome these difficulties by combining wholemount immunofluorescence for early lymphatic markers, Prox1 and Lyve-1, with optical clearing and three-dimensional imaging of mouse embryonic kidneys. All animal experiments were approved by the UK Home Office. Our data shows that the majority of renal lymphatic vessels arise at embryonic day 14.5 as an endothelial cell plexus restricted to the renal hilum. These endothelial cells extend towards the renal cortex, express classic markers of lymphatic endothelium, and form lumina later in nephrogenesis. We also identified a novel population of Prox1^{+}/Lyve-1^{+} cell clusters in the developing kidney, anatomically distinct from the main lymphatic tree, which co-express FoxD1^{+}, a marker of the renal interstitial stroma. *Cre* recombinase based lineage tracing using the pan-endothelial *Tie2* promoter labelled the majority of the renal lymphatic tree, but not Prox1^{+}/Lyve-1^{+} cell clusters. These findings suggest that renal lymphatic vessels arise heterogeneously, by both lymphangiogenesis and lymphvasculogenesis, from endothelial and non-endothelial contributions. Elucidating novel mechanisms for renal-specific lymphatic development may serve as a foundation for lymphatic-based therapies for kidney disease.