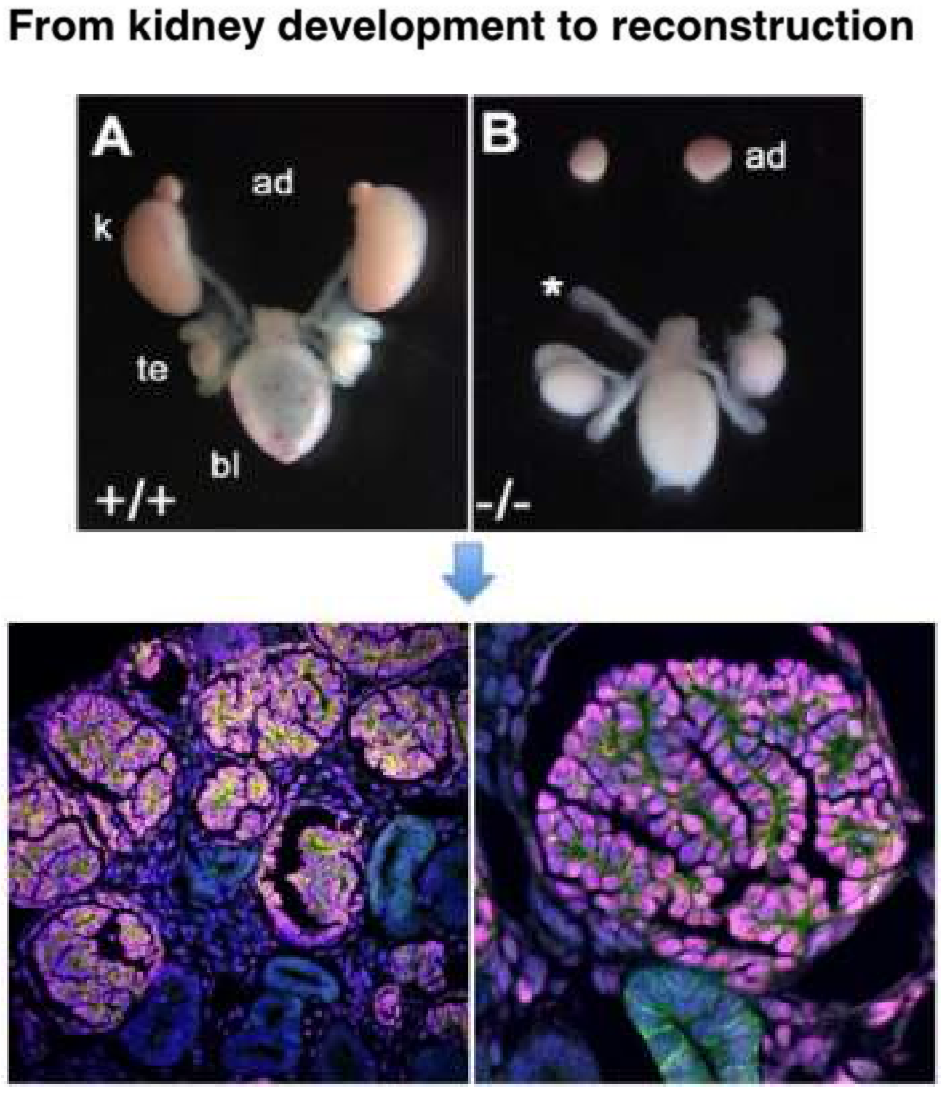
腎臓発生分野
Department of Kidney Development

腎臓は生命維持に必須の臓器であるが、一旦機能が障害されるとその回復は困難である。腎臓発生分野では、1) 遺伝子改変マウスを使って腎臓発生機構を解明し、その知見に立脚して、2) ヒト iPS 細胞からの腎臓系譜の誘導、さらには臓器としての高次構造の再構築に取り組んでいる。

The kidney is an important organ for life, but it never regenerates once impaired. Our research interests are as follows: 1) elucidation of molecular mechanisms in kidney development by using genetically engineered mice; 2) induction of kidney lineages from human iPS cells to reconstitute the higher-order organ structures, based on the findings on developmental nephrology.



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腎不全による人工透析患者数は国内に 32 万人、その医療費は年間 1.5 兆円を越えている。腎移植が腎不全の唯一の根治的治療だが、ドナー不足に悩まされている。このような現状の一方で、腎臓のような３次元臓器を作ることは極めて困難とされてきた。腎臓を作るには腎臓がどうやって発生するかを知ることが必要である。腎臓は後腎間葉と尿管芽という二つの胎児組織の相互作用によって形成され、前者から糸球体や尿細管というネフロン（腎臓の最小機能単位）が、後者からは集合管が形成される。我々はカエル及びマウスを用いて、後腎間葉に発現する核内因子 Sall1 が腎臓発生に必須なこと、後腎間葉中に Sall1 陽性の多能性ネフロン前駆細胞が存在することを報告してきた (Development 2001&2006, J Am Soc Nephrol 2014)。そしてこのネフロン前駆細胞の正しい起源を同定することによって、マウス ES 細胞及びヒト iPS 細胞からネフロン前駆細胞を経由して糸球体および尿細管構造を高効率に誘導することに成功した (Cell Stem Cell 2014)。ヒト iPS 細胞由来のネフロン前駆細胞をマウスに移植すると、ヒト糸球体がマウス血管内皮と接続し、糸球体上皮（ポドサイト）の成熟も進んだ (J Am Soc Nephrol 2016)。ネフロン前駆細胞の試験管内増幅も達成しつつある (Cell Rep 2016)。さらに尿管芽の誘導にも成功し、マウス腎臓の高次構造を試験管内で再現した（Cell Stem Cell 2017）。これらの成果は、遺伝性腎疾患の試験管内での病態再現に貢献するとともに、多能性幹細胞から腎臓そのものの構築につながることが期待される。

1. ネフロン前駆細胞と腎臓組織の誘導

ネフロン前駆細胞は、通説の前方中間中胚葉からではなく、胎生 8.5 日の後方未分化中胚葉から後方中間中胚葉を経て形成されることを見出した。このネフロン前駆細胞の正しい起源の同定を基盤にして、マウス ES 細胞からネフロン前駆細胞を誘導する方法を確立した。誘導されたネフロン前駆細胞は、試験管内で多数の糸球体および尿細管を形成することができた。さらにヒト iPS 細胞からも、ほぼ同じプロトコールを用いて、３次元の糸球体および尿細管構造を高効率に誘導することに成功した (Cell Stem Cell 2014)。

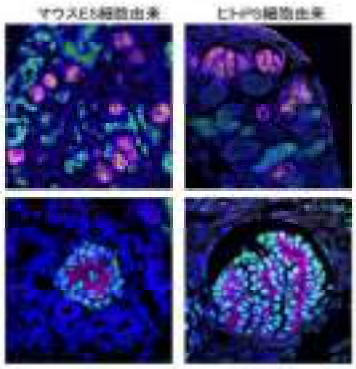

図1：マウスES細胞及びヒトiPS細胞から誘導した腎臓組織

2. ヒト iPS 細胞由来の糸球体への血管接続

糸球体ポドサイトに特徴的な Nephrin の遺伝子座に GFP を挿入したヒト iPS 細胞を作製し、誘導したヒトポドサイトが生体内のポドサイトに特徴的な遺伝子群を発現することを明らかにした。さらに iPS 細胞由来のネフロン前駆細胞をマウス腎臓に移植すると、ヒトの糸球体にマウスの血管が取り込まれ、ポドサイトは特徴的なろ過膜構造を形成した。試験管内で作ったヒト腎臓糸球体が移植により血管とつながって、さらに成熟することを示したものである。

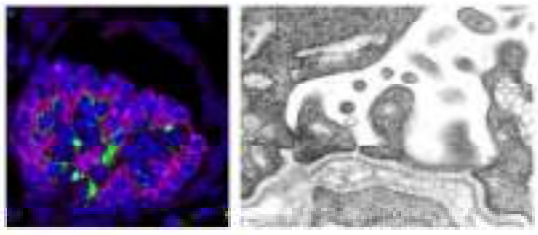
図2：ヒトiPS細胞由来糸球体への血管の取り込み（左）と
ポドサイトの成熟促進（右）

3. ネフロン前駆細胞の試験管内増幅

ネフロン前駆細胞の試験管内増幅も部分的であるが達成した。ネフロン前駆細胞は腎臓ができあがる出生前後に消失してしまうが、LIF、

WNT 及び BMP を敢えて低い濃度で添加することによって、マウスの胎仔から単離したネフロン前駆細胞を試験管内で約 20 日間培養し、約 1,800 倍に増幅することに成功した（Cell Rep 2016）。この培養法をヒト iPS 細胞から誘導したネフロン前駆細胞に適用したところ、8 日間で 4 倍に増幅し、増えた細胞は糸球体と尿細管を形成した。よって、液性因子を最適な濃度で組み合わせることにより、ネフロン前駆細胞の生存期間を延長して、増やすことが可能になった。現在、より長期にわたって増幅できる培養法を開発中である。

4. 尿管芽の誘導と腎臓高次構造の再構築

ネフロン同士の接続や配置といった腎臓の高次構造の形成に特に重要な役割を果たす「尿管芽」に注目し、多能性幹細胞から尿管芽を誘導する方法の開発を行い、腎臓の高次構造の再現に成功した (Cell Stem Cell 2017)。

マウス ES 細胞から誘導した尿管芽の機能性を確認するために、マウス ES 細胞由来のネフロン前駆細胞、及びマウス胎仔由来の間質前駆細胞と混ぜ合わせて器官培養を行った。in vivo の尿管芽には、①樹状分岐形成能力、②分岐の先端でネフロン前駆細胞からネフロンを分化させる能力、③ネフロン前駆細胞の一部を前駆細胞のまま維持させる能力の 3 つの機能的特徴があることが知られている。検証の結果、1 つの誘導尿管芽から、6〜7 世代におよぶ樹状分岐が形成されること、その各先端に分化したネフロンが接続すること、さらに未分化なネフロン前駆細胞も維持されることが確認され、典型的な胎児腎臓の高次構造が再現されることが確認された（図 3，4）。これらの結果は、マウス ES 細胞から誘導した尿管芽が生体の尿管芽と同様の機能特性を備えていること、それぞれ異なる方法で誘導した前駆細胞を組み合わせることで臓器の高次構造を再構築できることを示している。ヒト iPS 細胞からもゲル内で分岐する尿管芽が誘導できており、ヒト腎臓の高次構造構築に向けて研究を進めている。

図3：マウスES細胞から構築した腎臓の高次構造

5. 腎臓発生に必須な遺伝子群の同定

ネフロン前駆細胞特異的 Sall1 ノックアウトマウスを作成し、Sall1 がネフロン前駆細胞の維持に必須であることとその分子機構を明らかにした (J Am Soc Nephrol 2014)。Dullard のネフロン前駆細胞特異的ノックアウトマウスは、生後数週内に腎臓の中心部が細胞死によって空洞化した。このことから Dullard が BMP シグナルを抑制して適度なレベルに保つことが、出生後の腎臓の維持に必須であることが明らかになった (Nat Commun 2013)。転写因子 Six1/Six4 は、腎臓欠損に加えて、生殖腺の発生と性決定に必須であった (Dev Cell 2013)。非筋肉型ミオシン重鎖 IIA, B をコードする Myh9/10 の欠失は、ネフロン前駆細胞系譜ではネフロンの形成異常を、尿管芽では上皮の管腔への逸脱を起こした(J Am Soc Nephrol 2015, Dev Biol 2018)。転写因子 Isl1 の欠失は、尿管芽の異所的形成によって腎臓の無形成や尿管拡張を起こした（J Am Soc Nephrol 2013）。これらの知見は、腎臓誘導の際に有用な知見となった。

6. 共同研究

Sall1 が予想外に脳内ミクログリアの特異的マーカーであることが見出された (Nat Immunol 2016)のを契機に、Sall1CreER, Sall1GFP マウスを多くの研究室に提供した。

また Sall4 が ES 細胞及び生殖細胞に必須であることを報告していた（Development 2006, Stem Cells 2009&2015）が、ES 細胞における Sall4 の分子機構の解析も更に進んだ（Genes&Dev 2016, Mol Cell 2016, Development 2016）。

1. Redefining the in vivo origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells (Cell Stem Cell 2014)

Recapitulating three-dimensional (3D) structures of complex organs, such as the kidney, from pluripotent stem cells (PSCs) is a major challenge. Here, we define the developmental origins of the metanephric mesenchyme (MM), which generates most kidney components. Unexpectedly, we find that posteriorly located T^+ MM precursors are developmentally distinct from $Osr1^+$ ureteric bud progenitors during the postgastrulation stage, and identify phasic Wnt stimulation and stage-specific growth factor addition as molecular cues that promote their development into the MM. We then use this information to derive MM from PSCs. These progenitors reconstitute the 3D structures of the kidney in vitro, including glomeruli with podocytes and renal tubules with proximal and distal regions and clear lumina. Furthermore, the glomeruli are efficiently vascularized upon transplantation. Thus, by reevaluating the developmental origins of metanephric progenitors, we have provided key insights into kidney specification in vivo and taken important steps toward kidney organogenesis in vitro.

2. Human induced pluripotent stem cell-derived podocytes mature into vascularized glomeruli upon experimental transplantation (J Am Soc Nephrol 2016)

Glomerular podocytes express proteins, such as nephrin, that constitute the slit diaphragm, thereby contributing to the filtration process in the kidney. Glomerular development has been analyzed mainly in mice, whereas analysis of human kidney development has been minimal because of limited access to embryonic kidneys. We previously reported the induction of three-dimensional primordial glomeruli from human induced pluripotent stem (iPS) cells. Here, using transcription activator–like effector nuclease-mediated homologous recombination, we generated human iPS cell lines that express green fluorescent protein (GFP) in the NPHS1 locus, which encodes nephrin, and we show that GFP expression facilitated accurate visualization of nephrin-positive podocyte formation in vitro. These induced human podocytes exhibited apicobasal polarity, with nephrin proteins accumulated close to the basal domain, and possessed primary processes that were connected with immature slit diaphragm–like structures. Microarray analysis of sorted iPS cell–derived podocytes identified well-conserved marker gene expression previously shown in mouse and human podocytes in vivo. Furthermore, we developed a novel transplantation method using spacers that release the tension of host kidney capsules, thereby allowing the effective formation of glomeruli from human iPS cell–derived nephron progenitors. The human glomeruli were vascularized with the host mouse endothelial cells, and iPS cell–derived podocytes with numerous cell processes and the slit-diaphragm-like structures accumulated around the fenestrated endothelial cells. Therefore, the podocytes generated from iPS cells retain the podocyte-specific molecular and structural features, which will be useful for dissecting human glomerular development and diseases.

3. Selective in vitro propagation of nephron progenitors derived from embryos and pluripotent stem cells (Cell Rep 2016)

Nephron progenitors in the embryonic kidney propagate while generating differentiated nephrons. However, in mice, the progenitors terminally differentiate shortly after birth. Here, we report a method to selectively expand nephron progenitors in vitro in an undifferentiated state. Combinatorial and concentration-dependent stimulation with LIF, FGF2/9, BMP7, and a WNT agonist is critical for expansion. The purified progenitors proliferated beyond the physiological limits observed in vivo, both for cell numbers and lifespan. Neonatal progenitors were maintained for a week, while progenitors from embryonic day 11.5 expanded 1800-fold for nearly 20 days and still reconstituted three-dimensional nephrons containing glomeruli and renal tubules. Furthermore, progenitors generated from mouse embryonic stem cells and human induced pluripotent cells could be expanded with retained nephron-forming potential. Thus, we have established in vitro conditions to promote propagation of nephron progenitors, which will be essential for dissecting the mechanisms of kidney organogenesis and for regenerative medicine.

4. Higher-order kidney organogenesis from pluripotent stem cells (Cell Stem Cell 2017)

Organogenesis generates higher-order structures containing functional subunits, connective components, and progenitor niches. Despite recent

advances in organoid-based modeling of tissue development, recapitulating these complex configurations from pluripotent stem cells (PSCs) has remained challenging. In this study, we report assembly of kidney organoids that recapitulate embryonic branching morphogenesis. By studying the distinct origins and developmental processes of the ureteric bud, which contains epithelial kidney progenitors that undergo branching morphogenesis and thereby plays a central role in orchestrating organ geometry, and neighboring mesenchymal nephron progenitors, we established a protocol for differential induction of each lineage from mouse and human PSCs. Importantly, reassembled organoids developed the inherent architectures of the embryonic kidney, including the peripheral progenitor niche and internally differentiated nephrons that were interconnected by a ramified ureteric epithelium. This selective induction and reassembly strategy will be a powerful approach to recapitulate organotypic architecture in PSC-derived organoids.

5. Identification of essential genes for kidney development by using knockout mice

We report that Sall1 deletion in Six2-positive nephron progenitors results in severe progenitor depletion and apoptosis of the differentiating nephrons in mice. Sall1 maintains nephron progenitors and their derivatives by a unique mechanism, which partly overlaps but is distinct from that of Six2. (J Am Soc Nephrol 2014). We also report that Dullard keeps BMP signaling at an appropriate level, which is required for nephron maintenance in the postnatal period. (Nat Commun 2013). Loss of Six1 and Six4, but neither alone, results in a male-to-female sex reversal phenotype in XY-mutant gonads accompanied by a failure in *Sry* activation. (Dev Cell 2013). We also report deletion of non-muscle myosin II genes in nephron progenitor or ureteric bud lineages. (J Am Soc Nephrol 2015, Dev Biol 2017), as well as deletion of Isl1 (J Am Soc Nephrol 2013).

6. Collaborations

We report that Sall1 is a specific marker of microglila (Nat Immunol 2016), and roles of Sall4 in maintenance of ES cells (Genes&Dev 2016, Mol Cell 2016, Development 2016).

論文目録　Publications

1. Hayata T, Chiga M, Edura Y, Asashima M, Katabuchi H, Nishinakamura R, Noda M. Dullard deficiency causes hemorrhage in the adult ovarian follicles. ***Genes Cells*** 1074:209-215, 2018.

2. Nishinakamura R. The era of human developmental nephrology. ***J Am Soc Nephrol*** 29(3): 705-706, 2018.

3. Taguchi A and Nishinakamura R. Higher-order kidney organogenesis from pluripotent stem cells. ***Cell Stem Cell*** 21(6): 730-746, 2017.

4. Susman MW, Karuna EP, Kunz RC, Gujral TS, Cantú AV, Choi SS, Jong BY, Okada K, Scales MK, Hum J, Hu LS, Kirschner MW, Nishinakamura R, Yamada S. Kinesin superfamily protein Kif26b links Wnt5a-Ror signaling to the control of cell and tissue behaviors in vertebrates. ***Elife*** 6: e26509, 2017.

5. Hosoe-Nagai Y, Hidaka T, Sonoda A, Sasaki Y, Yamamoto-Nonaka K, Seki T, Asao R, Tanaka E, Trejo JAO, Kodama F, Takagi M, Tada N, Ueno T, Nishinakamura R, Tomino Y, Asanuma K. Re-expression of Sall1 in podocytes protects against adriamycin-induced nephrosis. ***Lab Invest*** 97(11):1306-1320, 2017.

6. Kaku Y, Taguchi A, Tanigawa S, Haque F, Sakuma T, Yamamoto T and Nishinakamura R. PAX2 is dispensable for in vitro nephron formation from human induced pluripotent stem cells. ***Sci Rep*** 7(1): 4554, 2017.

7. Nishinakamura R and Takasato M. Human development, heredity and evolution. ***Development*** 144(12): 2099-2103, 2017.

8. Haque F, Kaku Y, Fujimura S, Ohmori T, Adelstein RS, and Nishinakamura R. Non-muscle myosin II deletion in the developing kidney causes ureter-bladder misconnection and apical extrusion of the nephric duct lineage epithelia. ***Dev Biol*** 427(1):121-130, 2017.

9. Yoshimura Y, Taguchi A, and Nishinakamura R. Generation of a three-dimensional kidney structure from pluripotent stem cells. ***Methods Mol Biol*** 1597: 179-193, 2017.

10. Xiong J, Zhang Z, Chen J, Huang H, Xu Y, Ding X, Zheng Y, Nishinakamura R, Xu GL, Wang H, Chen S, Gao S, Zhu B. Cooperative action between SALL4A and TET proteins in stepwise oxidation of 5-methylcytosine. ***Mol Cell*** 64(5):913-925, 2016.

11. Buttgereit A, Lelios I, Yu X, Vrohlings M, Krakoski NR, Gautier EL, Nishinakamura R, Becher B, Greter M. Sall1 is a transcriptional regulator defining microglia identity and function. ***Nat Immunol*** 17(12):1397-1406, 2016.

12. Tanigawa S and Nishinakamura R. Expanding nephron progenitors in vitro: a step toward regenerative medicine in nephrology. ***Kidney Int*** 90(5): 925-927, 2016.

13. Miller A, Ralser M, Kloet SL, Loos R, Nishinakamura R, Bertone P, Vermeulen M, and Hendrich B. Sall4 controls differentiation of pluripotent cells independently of the Nucleosome Remodelling and Deacetylation (NuRD) complex. ***Development*** 143(17): 3074-3084, 2016.

14. Sasaki T, Tanaka Y, Kulkeaw K, Yumine-Takai A, Tan KS, Nishinakamura R, Ishida J, Fukamizu A, and Sugiyama D. Embryonic intra-aortic clusters undergo myeloid differentiation mediated by mesonephros-derived CSF1 in mouse. ***Stem Cell Rev*** 12(5):530-542, 2016.

15. Tanigawa S, Taguchi A, Sharma N, Perantoni AO, and Nishinakamura R. Selective in vitro propagation of nephron progenitors from embryos and pluripotent stem cells. ***Cell Rep*** 15(4): 801-813, 2016

16. Novo CL, Tang C, Ahmed K, Djuric U, Fussner E, Mullin NP, Morgan NP, Hayre J, Sienerth AR, Elderkin S, Nishinakamura R, Chambers I, Ellis J, Bazett-Jones DP, and Rugg-Gunn PJ. The pluripotency factor Nanog regulates pericentromeric heterochromatin organization in mouse embryonic stem cells. ***Genes Dev*** 30(9):1101-1115, 2016.

17. Morita Y, Andersen P, Hotta A, Tsukahara Y, Sasagawa N, Hayashida N, Koga C, Nishikawa M, Saga Y, Evans S, Koshiba-Takeuchi K, Nishinakamura R, Yoshida Y, Kwon C, and Takeuchi JK. Sall1 transiently marks undifferentiated heart precursors and regulates their fate. ***J Mol Cell Cardiol*** 92: 158-162, 2016.

18. Nishinakamura R, Sharmin S, and Taguchi A. Induction of nephron progenitors and glomeruli from human pluripotent stem cells. ***Pediatr Nephrol*** 32(2):195-200, 2016.

19. Nishinakamura R. Stem cells and renal development in 2015: Advances in generating and maintaining nephron progenitors. ***Nat Rev Nephrol*** 12(2):67-68, 2016.

20. Sharmin S, Taguch A, Kaku Y, Yoshimura Y, Ohmori T, Sakuma T, Mukoyama M, Yamamoto T, Kurihara H, and Nishinakamura R. Human induced pluripotent stem cell-der4ived podocytes mature into vascularized glomeruli upon experimental transplantation. ***J Am Soc Nephrol*** 27(6):1778-1791, 2016.

21. Ohmori T, Tanigawa S, Kaku Y, Fujimura S, and Nishinakamura R. Sall1 in renal stromal progenitors non-cell autonomously restricts the excessive expansion of nephron progenitors. ***Sci Rep*** 5: 15676, 2015.

22. Tanigawa S, Sharma N, Hall MD, Nishinakamura R, Perantoni AO. Preferential propagation of competent SIX2+ nephronic progenitors by LIF/ROCKi treatment of the metanephric mesenchyme. ***Stem Cell Reports*** 5(3):435-47, 2015.

23. Sweetwyne MT, Gruenwald A, Niranjan T, Nishinakamura R, Strobl LJ, Susztak K. Notch1 and Notch2 in podocytes play differential roles during diabetic nephropathy development. ***Diatetes*** 64(12):4099-4111, 2015.

24. Escobar D, Hepp MI, Farkas C, Campos T, Sodir NM, Morales M, Álvarez CI, Swigart L, Evan GI, Gutiérrez JL, Nishinakamura R, Castro AF, Pincheira R. Sall2 is required for proapoptotic Noxa expression and genotoxic stress-induced apoptosis by doxorubicin. ***Cell Death Dis.*** 6:e1816, 2015.

25. Hirsch S, El-Achkar TM, Robbins L, Basta J, Heitmeier MR, Nishinakamura R, Rauchman M. A mouse model of Townes-Brocks syndrome expressing a truncated mutant Sall1 protein is protected from acute kidney injury. ***Am J Physiol Renal Physiol.*** 309(10):F852-863, 2015.

26. Akiyama R, Kawakami H, Wong J, Oishi I, Nishinakamura R, Kawakami Y. Sall4-Gli3 system in early limb progenitors is essential for the development of limb skeletal elements. ***Proc Natl Acad Sci USA*** 112(16): 5075-5080, 2015.

27. Taguchi A and Nishinakamura R. Nephron reconstitution from pluripotent stem cells. ***Kidney Int*** 87(5):894-900, 2015

28. Suzuki K, Numata T, Suzuki H, Raga DD, Ipulan LA, Yokoyama C, Matsushita S, Hamada M, Nakagata N, Nishinakamura R, Kume S, Takahashi S, and Yamada G. Sexually dimorphic expression of Mafb regulates masculinization of the embryonic urethral formation. ***Proc Natl Acad Sci USA*** 111(46): 16407-16412, 2014.

29. Yamaguchi YL, Tanaka SS, Kumagai M, Fujimoto Y, Terabayashi T, Matsui Y, and Nishinakamura R. Sall4 is essential for mouse primordial germ cell specification by suppressing somatic program genes. ***Stem Cells*** 33(1): 289-300, 2015.

30. Recuenco MC, Ohmori T, Tanigawa S, Taguchi A, Fujimura S, Conti MA, Wei Q, Kiyonari H, Abe T, Adelstein RS and Nishinakamura R. Non-muscle myosin II regulates the morphogenesis of metanephric mesenchyme-derived immature nephrons. ***J Am Soc Nephrol*** 26(5):1081-1091, 2015.

31. Hayata T, Ezura Y, Asashima M, Nishinakamura R, Noda M. Dullard/Ctdnep1 regulates endochondral ossification via suppression of TGF-β signaling. ***J Bone Miner Res.*** 30(2):318-329, 2015.

32. Kanda S, Tanigawa S, Ohmori T, Taguchi A, Kudo K, Suzuki Y, Sato Y, Hino S, Sander M, Perantoni AO, Sugano S, Nakao M, and Nishinakamura R. Sall1 maintains nephron progenitors and nascent nephrons by acting as both an activator and a repressor. ***J Am Soc Nephrol*** 25(11): 2584-2595, 2014.

33. Tanaka SS and Nishinakamura R. Regulation of male sex determination: genital ridge formation and Sry activation in mice. ***Cell Mol Life Sci*** 71(24): 4781-4802, 2014.

34. Nishita M, Qiao S, Miyamoto M, Okinaka Y, Yamada M, Hashimoto R, Iijima K, Otani H, Hartmann C, Nishinakamura R, and Minami Y. Role of Wnt5a-Ror2 signaling in morphogenesis of the metanephric mesenchyme during ureteric budding. ***Mol Cell Biol*** 34(16): 3096-3105, 2014.

35. Ipulan LA, Suzuki K, Sakamoto Y, Murashima A, Imai Y, Omori A, Nakagata N, Nishinakamura R, Valasek P, and Yamada G. Non-myocytic androgen receptor regulates the sexually dimorphic development of the embryonic bulbocavernosus muscle. ***Endocrinology.*** 155(7): 2467-2479, 2014.

36. Chiga M, Ohmori T, Ohba T, Katabuchi H, and Nishinakamura R. Preformed Wolffian duct regulates Müllerian duct elongation independently of canonical Wnt signaling or Lhx1 expression. ***Int J Dev Biol*** 58(9):663-668, 2014.

37. Murashima A, Akita H, Okaszawa M, Kishigami S, Nakagata N, Nishinakamura R, and Yamada G. Midline-derived Shh regulated mesonephric tubule formation through the paraxial mesoderm. ***Dev Biol*** 386(1): 216-226, 2014.

38. Taguchi A, Kaku Y, Ohmori T, Sharmin S, Ogawa M, Sasaki H, and Nishinakamura R. Redefining the in vivo origin of metanephric nephron progenitors enables generation of complex kidney structures from pluripotent stem cells. ***Cell Stem Cell*** 14(1): 53-67, 2014.

39. Nishinakamura R and Sakaguchi M. BMP signaling and its modifiers in kidney development. ***Pediatr Nephrol*** 29(4): 681-686, 2014.

40. Liu L, Souto J, Liao W, Jiang Y, Li Y, Nishinakamura R, Huang S, Rosengart T, Yang VW, Schuster M, Ma Y, and Yang J. Histone lysine-specific demethylase 1 (LSD1) protein Is involved in Sal-like protein 4 (SALL4)-mediated transcriptional repression in hematopoietic stem cells. ***J Biol Chem*** 288(48):34719-34728, 2013.

41. Fujimoto Y, Tanaka SS, Yamaguchi YL, Kobayashi H, Kuroki S, Tachibana M, Shinomura M, Kanai Y, Morohashi K, Kawakami K, and Nishinakamura R. Homeoprotein Six1 and Six4 regulate male sex determination and mouse gonadal development. ***Dev Cell*** 26(4): 416-430, 2013.

42. Toyoda D, Taguchi A, Chiga M, Ohmori T, and Nishinakamura R. Sall4 is expressed in the caudal Wolffian duct and the ureteric bud, but dispensable for kidney development. ***PLoS One*** 8(6): e68508. 2013.

43. Kaku Y, Ohmori T, Kudo K, Fujimura S, Suzuki S, Evans SM, Kawakami K, and Nishinakamura R. *Islet1* deletion causes mouse kidney agenesis and hydroureter resembling CAKUT. ***J Am Soc Nephrol*** 24(8): 1242-1249, 2013.

44. Tanaka SS, Nakane A, Yamaguchi YL, Terebayashi T, Abe T, Nakao K, Asashima M, Steiner KA, Tam PP, and Nishinakamura R. Dullard/Ctdnep1 modulates WNT signaling activity for the formation of primordial germ cells in the mouse embryo. ***PLoS One*** 8(3) e57428, 2013.

45. Sakaguchi M, Sharmin S, Taguchi A, Ohmori T, Fujimura S, Abe T, Kiyonari H, Komatsu Y, Mishina Y, Asashima M, Araki E, and Nishinakamura R. The phosphatase Dullard negatively regulates BMP signalling and is essential for nephron maintenance after birth. ***Nat Commun*** 4: 1398, 2013.

46. Morimoto M, Nishinakamura R, Saga Y, and Kopan R. Different assemblies of Notch receptors coordinate the distribution of the major bronchial Clara, ciliated and neuroendocrine cells. ***Development*** 139(23):4365-73, 2012.

47. Terabayashi T, Sakaguchi M, Shinmyozu K, Ohshima T, Johjima A, Ogura T, Miki H, and Nishinakamura R. Phosphorylation of Kif26b promotes its polyubiquitination and subsequent proteasomal degradation during kidney development. ***PLoS One*** 7(6):e39714, 2012.

48. Usui J, Kobayashi K, Yamaguchi T, Knisely AS, Nishinakamura R and Nakauchi H. Generation of kidney from pluripotent stem cells via

blastocyst complementation. ***Am J Pathol*** 80(6): 2417-2426, 2012.

49. Hobbs RM, Fagoonee S, Papa A, Webster K, Altruda F, Nishinakamura R, Chai L, and Pandolfi PP. Functional antagonism between Sall4 and Plzf define germline progenitors. ***Cell Stem Cell*** 10(3): 284-298, 2012.

50. Harrison SJ, Nishinakamura R, Jones KR, and Monaghan AP. Sall1 regulates cortical neurogenesis and laminar fate specification, implications for neural abnormalities in Townes-Brocks Syndrome. ***Dis Model Mech*** 5(3):351-365, 2012.

著書・総説目録　Publications

1. Taguchi A and Nishinakamura R. Early kidney specification and its recapitulation by pluripotent stem cells. (Chapter 9) In: Organ regeneration based on developmental biology (edited by Takashi Tsuji) Springer 153-177, 2017.

2. 田中悦子、西中村隆一　「腎臓の機能的立体化とその医学応用への可能性」医学のあゆみ（医歯薬出版）　264(8):659-663, 2018.

3. 倉岡将平、西中村隆一　「発生学に基づく腎臓の再構築」　移植　52(4-5): 318-324, 2017.

4. 長沼英和、太口敦博、西中村隆一　「腎臓の発生に関する最新の知見」小児外科(東京医学社) 49(9):857-863, 2017.

5. 西中村隆一「腎臓を創る 」日本内科学会雑誌　106(9): 1936-1940, 2017.

6. 太口敦博、西中村隆一　「腎オルガノイド x Developmental Biology」オルガノイド 4.0 時代　実験医学（羊土社）35(16): 2695-2702, 2017.

7. 西中村隆一「腎再生医療の現状と課題：Overview 」　腎臓内科・泌尿器科　5(6): 529-531, 2017.

8. 谷川俊祐、西中村隆一　「ネフロン前駆細胞の試験管内誘導と増幅培養法の開発」腎臓発生学と再生医学への応用（企画：西中村隆一）医学のあゆみ（医歯薬出版）257(11):1127-1132, 2016.

9. 太口敦博、西中村隆一　「三次元の腎臓を創る」腎臓のサイエンス実験医学（羊土社）34(8):1228-1235, 2016.

10. 西中村隆一　「腎臓にサイエンスはあるか？」腎臓のサイエンス（企画：西中村隆一）実験医学（羊土社）34(8):1216-1221, 2016.

11. 西中村隆一「本物の臓器を作るには？」Bio Clinca 31(3): 223, 2016.

12. Nishinakamura R and Taguchi A. From development to regeneration: Kidney reconstitution in vitro and in vivo. (Chapter 34) In: Kidney development, disease, repair, and regeneration (edited by Melissa H Little) Academic Press 2015: 463-472

13. 永松翔、太口敦博、西中村隆一　「iPS 細胞から糸球体を創る」腎と透析（東京医学社）　79(6): 935-940, 2015

14. 西中村隆一　「三次元腎臓組織の試験管内構築」発達腎研究会誌　23(1): 3-7, 2015.

15. 村上陽一、太口敦博、西中村隆一「多能性幹細胞を用いた腎臓再生への展望」日本臨牀 73(5), 175-178, 2015.

16. 太口敦博、西中村隆一　「発生機構の解析から挑む iPS 細胞からの腎臓三次元再構築」　医学の歩み 253(2), 193-201, 2015.

17. 賀来祐介、太口敦博、西中村隆一「3 次元腎臓組織の試験管内誘導」三次元ティッシュエンジニアリング技術最前線 253-258, 2015.

18. 吉村仁宏、太口敦博、西中村隆一「多能性幹細胞からの立体的腎臓組織の作製」月刊糖尿病 7(3), 42-49, 2015.

19. 谷川俊祐、西中村隆一「腎臓発生学の進展と再生医学への応用」日本腎臓学会誌 57(1): 233-240, 2015.

20. 谷川俊祐、西中村隆一「腎臓の発生・再生医学：最近の進歩」Annual Review 腎臓 2015（中外医学社）58-67, 2015.

21. 太口敦博　西中村隆一　新しい腎臓発生モデルと試験管内腎臓作製への展望―下半身臓器の誘導に必要な「体軸幹細胞」という概念―　腎と透析　77(6): 925-932, 2014.

22. 太口敦博、西中村隆一　新しい腎臓発生モデルの構築と多能性幹細胞を用いた腎臓再生への展開　内分泌・糖尿病・代謝内科 39(2): 173-180, 2014.

23. 豊田大地、西中村隆一「再生医療の現状と未来」腎疾患・透析　最新の治療（南江堂）1-5, 2014.

24. 太口敦博、西中村隆一　腎臓の初期発生の新たなモデルと多能性幹細胞からの腎臓の組織の構築　ライフサイエンス新着論文レビュー　Jan 14, 2014.

25. 谷川俊祐、西中村隆一「腎発生学の進展と再生医療の展望」Annual Review 腎臓 2014（中外医学社）105-111, 2014.

26. 西中村隆一、谷川俊祐　「腎臓の発生とその制御機構」腎と透析（東京医学社）75(6): 831-834, 2013.

27. 西中村隆一　「腎臓再生の現状と展望」幹細胞研究と再生医療（中内啓光編集）（南山堂）201-207, 2013.

28. 田中聡、藤本由佳、西中村隆一　マウスにおいて転写因子Six1およびSix4は生殖腺の形成と雄への分化を制御する　ライフサイエンス新着論文レビュー　Sep 17, 2013.

29. 西中村隆一　「１枚の写真館― 腎臓がないマウス」細胞工学（秀潤社）32(4):379, 2013.

30. 賀来祐介、西中村隆一「腎臓発生の分子機構と発生への展望」生化学（日本生化学会）84(12): 985-993, 2012.

31. 西中村隆一「ネフロン前駆細胞からみた腎臓発生機構」腎と透析（東京医学社）73(3): 457-461, 2012.

32. 太口敦博、西中村隆一「多能性幹細胞から腎臓細胞への誘導」　内分泌・糖尿病・代謝内科（科学評論社）35(2): 125-129, 2012.

学会・研究集会 発表目録　Meeting Presentations

1. 西中村隆一　試験管内で腎臓を創る　第52回新潟腎カンファランス 2018.3.29　新潟（特別講演）.

2. Tanigawa S, Kaku Y, Naganuma H, Taguchi A and Nishinakamura R. Maintenance and Propagation of Human Nephron Progenitors In Vitro. Key Forum: The 3rd international symposium on stem cell traits and developmental systems. 2018.1.12 熊本（ポスター発表）.

3. Nishinakamura R and Taguchi A. Building the kidney from pluripotent stem cells. Key Forum: The 3rd international symposium on stem cell traits and developmental systems. 2018.1.12 熊本　（指定発表）.

4. 西中村隆一　試験管内で腎臓を創る　第10回 YORAN Jung Niere の会　2017.12.23 福岡（特別講演）.

5. 村上陽一、長沼英和、谷川俊祐、西中村隆一　マウス胎生期の腎臓における血管内皮細胞の分布と遺伝子発現 心血管代謝週間 CVMW2017　2017.12.8　(ポスター発表)

6. 谷川俊祐、賀来祐介、太口敦博、西中村隆一　３次元組織形成能を持つヒト iPS 由来

腎臓ネフロン前駆細胞の増幅培養法の確立　生命科学系学会合同年次大会 ConBio 2017　2017.12.8　神戸（口頭発表、ポスター発表）

7. 西中村隆一　均一な前駆細胞誘導に基づく不均一な３次元腎臓構造の形成　生命科学系学会合同年次大会 ConBio 2017 2017.12.8　神戸（指定発表）

8. 西中村隆一　試験管内で腎臓を創る　第 50 回九州人工透析研究会総会 2017.12.3 福岡（特別講演）

9. 西中村隆一　幹細胞から腎臓を創る　第 39 回日本小児腎不全学会 2017.9.22　淡路島（特別講演）

10. 西中村隆一　発生学を基盤に腎臓を創る Research PlaNet 2017　2017.6.25　京都（指定発表）

11. 西中村隆一　幹細胞から腎臓を創る　第 22 回　阿蘇腎フォーラム　2017.6.8　福岡（特別講演）

12. 西中村隆一　幹細胞から腎臓を創る　第 105 回日本泌尿器科学会総会 2017.4.22　鹿児島　（特別講演）

13. 西中村隆一 教育講演：腎臓を創る 第 114 回日本内科学会総会 2017.4.14 東京（指定講演）

14. Fahim Haque, Yusuke Kaku, and Nishinakmaura R. Non-muscle myosin II deletion in the developing kidney causes ureter-bladder misconnection and apical extrusion of the nephric duct lineage epithelia. 2017.5.10. 東京（口頭発表）

15. Nishinakamura R. Building the kidney from pluripotent stem cells CDB Symposium 2017. Mar 28, 2017. Kobe, Japan (invited oral presentation)

16. Taguchi A. and Nishinakamura R. Detailed Functional And Gene Expression profiling uncovers the early Wolffian duct maturation processes Joint Meeting of the German and Japanese Societies of Developmental Biologists. March 17, 2017. Kiel, Germany (oral and poster presentation).

17. 吉村仁宏、太口敦博、向山政志、西中村隆一　ネフロン前駆細胞からポドサイトへの選択的誘導シグナルの検討　ポドサイト研究会　2017.3.4　新潟（口頭発表、ポスター発表）

18. 西中村隆一　試験管内で腎臓を創る 第 44 回明石西神戸透析懇話会 2017.2.4　東京（指定講演）

19. 西中村隆一　発生学をもとに腎臓を創る 第 21 回分生研シンポジウム 2016.12.21 東京（指定講演）

20. 賀来祐介、太口敦博、谷川俊介、西中村隆一　ヒトネフロン前駆細胞からの腎臓上皮形成において PAX2 は不要である　日本分子生物学会　2016.12.2　横浜（ポスター発表）

21. 吉村仁宏、太口敦博、向山政志、西中村隆一　腎臓ポドサイト分化における時期特異的な Wnt シグナルの役割についての検討　日本分子生物学会　2016.11.30　横浜（ポスター発表）

22. 谷川俊祐、太口敦博、Sharma S, Perantoni AO、西中村隆一　３次元組織形成能を持つ腎臓ネフロン前駆細胞の増幅培養法の確立　日本分子生物学会　2016.11.30　横浜（ポスター発表）

23. Nishinakamura R. Complex 3D Kidney Structures from Pluripotent Stem Cells Kidney Week 2016 (American Society of Nephrology). Nov 18,2016. Chicago, USA　(invited oral presentation)

24. Nishinakamura R.　Recreating the kidney from pluripotent stem cells. 2nd Kumamoto IRCMS International Symposium　2016. 11.1 熊本（指定講演）

25. Nishinakamura R. Creating the kidney based on its developmental origin EMBO/EMBL symposium: Organoids: Modeling organ development and disease in 3D culture. Oct 14, 2016. Heidelberg, Germany ((invited oral presentation)

26. 西中村隆一　発生学を基盤に腎臓を創る 第 39 回日本高血圧学会　2016.10.2　仙台（指定講演）

27. 西中村隆一　発生学に基づく腎臓の再構築　第 46 回臨床分子形態学会 2016.9.23 熊本（招待講演）

28. 西中村隆一　Recreating human podocytes in the kidney 数理生物学会 2016.9.8 福岡（指定講演）

29. 谷川俊祐、西中村隆一　３次元組織形成能を持つネフロン前駆細胞の増幅培養法の確立　第７回分子腎臓フォーラム 2016.9.3　東京（口頭発表）

30. Nishinakamura R. Recreating the kidney. Santa Cruz Developmental Biology Meeting. Aug 15, 2016. Santa Cruz, USA (invited oral presentation)

31. 太口敦博、Sazia Sharmin、西中村隆一　iPS 細胞を用いた三次元の腎臓再生の現状と機能再建に向けた課題　日本サイトメトリー学会　2016.7.23　福岡（口頭発表）

32. 太口敦博、西中村隆一　腎臓の起源の理解と三次元再生への応用　日本透析医学会 2016.6.12　大阪（口頭発表）

33. 西中村隆一 発生学を基盤に腎臓を創る 第 27 回 NCGM 研究所セミナー 2016.5.20. 東京（招待講演）

34. Taguchi A. and Nishinakamura R. Detailed Functional And Gene Expression profiling uncovers the early Wolffian duct maturation processes International Society for Stem Cell Research (ISSCR) 2016. June 24, 2016. San Francisco, USA (poster presentation).

35. Tanigawa S, Taguchi A, Sharma S, Perantoni AO and Nishinakamura R. *In vitro* propagation of multipotent nephron progenitors from mouse embryo and embryonic stem cells. International Society for Stem Cell Research (ISSCR) 2016. June 24, 2016. San Francisco, USA (poster presentation).

36. Nishinakamura R. Programming stem cells toward the kidney. CiRA/ISSCR 2016 International Symposia. Pluripotency: From basic science to therapeutic applications. Mar 24, 2016. Kyoto, Japan (invited oral presentation)

37. 太口敦博、西中村隆一　細胞自律的かつ相互依存的な腎臓の形態形成過程の再現と応用への課題　第 15 回日本再生医療学会総会　シンポジウム　2016 年 3 月 19 日、大阪（口頭発表、西中村はオーガナイザー兼座長）

38. Haque F and Nishinakamura R. Cells lacking non-muscle myosin II in the developing kidney are expelled from the ureteric bud epithelia. 1st International Symposium on Cell Competition: Cell Competition in Development and Cancer. Sep 10, 2015. Kyoto, Japan (poster)

39. Tanigawa S, Sharma N, Michael HD, Nishinakamura R and Perantoni AO. Perpetuation of metanephric mesenchymal progenitors through LIF-induced Stat activation and stabilization of nuclear Six2 and Yap. 13th International Workshop on Developmental Nephrology. July 14, 2015. Salt Lake City, Utah, USA (poster)

40. Taguchi A. and Nishinakamura R. Dissecting the early kidney specification processes by utilizing in vivo lineage tracing and ex vivo directed differentiation approach. International Workshop on Developmental Nephrology. July 13, 2015. Salt Lake City, Utah, USA (poster)

41. Nishinakamura R. Programming stem cells toward the kidney. The 13th International Workshop on Developmental Nephrology. July 15, 2015. Salt Lake City, Utah, USA (invited oral presentation and organizer)

42. 西中村隆一　試験管内で腎臓を創る　第 27 回阪大医療組織工学フォーラム　2015 年 12 月 4 日、大阪（特別講演）

43. Nishinakamura R. Kidney progenitor expansion and generation of vascularized glomeruli from stem cells. 第 38 回日本分子生物学会年会 シンポジウム　2015 年 12 月 4 日、神戸　(口頭発表)

44. 谷川俊祐、太口敦博、Sharma N, Perantoni A.O, 西中村隆一　3 次元器官形成能を持つ腎臓ネフロン前駆細胞の増幅培養法 第 38 回日本分子生物学会年会　2015 年 12 月 4 日、神戸　(口頭発表、ポスター発表)

45. 賀来祐介、太口敦博、佐久間哲史、山本卓、西中村隆一　ネフロン前駆細胞特異的レポーター遺伝子を持つ iPS 細胞の樹立　第 38 回日本分子生物学会年会　2015 年 12 月 2 日、神戸（ポスター発表）

46. 太口敦博、Sharmin S、西中村隆一　ヒト iPS 細胞由来ポドサイトの遺伝子プロファイリングとその成熟化を促進する新規移植法の開発　第 38 回分子生物学会年会 2015 年 12 月 2 日、神戸（ポスター発表）

47. 西中村隆一　試験管内で腎臓を創る　臓器再生医療実用化を目指した研究戦略 2015 年 11 月 30 日、神戸（Keynote lecture）

48. 西中村隆一　試験管内で腎臓を創る　第 37 回生体膜と薬物の相互作用シンポジウム　2015 年 11 月 19 日、熊本（特別講演）

49. 西中村隆一　試験管内で腎臓を創る　千里ライフサイエンス新適塾　2015 年 11 月 2 日、千里（特別講演）

50. 太口敦博、西中村隆一　iPS 細胞からの腎臓組織作成法の確立と医療応用への展望 BioJapan2015　2015 年 10 月 14 日、横浜（口頭発表）

51. 西中村隆一　iPS 細胞から腎臓を創る CKD トータルケアレクチャー　2015 年 10 月 10 日、神戸（特別講演）

52. 西中村隆一　ヒト iPS 細胞からの３次元腎臓組織作成　第 26 回日本急性血液浄化学会　2015 年 10 月 9 日、東京（招聘講演）

53. 西中村隆一　試験管内で腎臓を創る　第 80 回日本泌尿器科学会東部総会　2015 年 9 月 27 日、東京（特別講演）

54. 西中村隆一　試験管内で腎臓を創る　第 20 回 Sendai Renal Research Seminar　2015 年 9 月 25 日、仙台（特別講演）

55. 西中村隆一　試験管内で腎臓を創る　第 39 回阿蘇シンポジウム　2015 年 7 月 31 日、熊本（口頭発表）

56. 西中村隆一　試験管内で腎臓を創る　第 19 回兵庫県腎疾患治療懇話会　2015 年 7 月 3 日、神戸（特別講演）

57. 西中村隆一　試験管内で腎臓を創る　内臓の life cycle 研究会　2015 年 6 月 21 日、神戸（口頭発表）

58. 西中村隆一　試験管内で腎臓を創る　第 50 回日本小児腎臓学会学術集会　2015 年 6 月 18 日、神戸（口頭発表）

59. Nishinakamura R. Programming stem cells to the kidney. ASN/JSN joint science symposium 第 58 回日本腎臓学会　2015 年 6 月 6 日、名古屋（口頭発表）

60. Nishinakamura R and Taguchi A. Creating the kidney in vitro. 第 48 回日本発生生物学会 2015 年 6 月 5 日、筑波（口頭発表）

61. Sharmin S, Taguchi A, Yoshimura Y, Ohmori T, Kaku Y, Sakuma T, Mukoyama M, Yamamoto T, Kurihara H, and Nishinakamura R. GFP-tagged glomerular podocytes generated from human iPS cells possess slit diaphragms and resemble the transcriptional state of podoctyes in vivo. 第 48 回日本発生生物学会　2015 年 6 月 3 日、筑波（口頭発表）

62. 西中村隆一　試験管内で腎臓を創る　第 25 回長崎障害者支援再生医療研究会 2015 年 5 月 26 日　長崎（特別講演）

63. 西中村隆一　ネフロン前駆細胞の転写因子ネットワークに基づく腎臓組織の試験管内誘導　日本臨床分子医学会　2015 年 4 月 10 日　京都（口頭発表）

64. 西中村隆一　試験管内で腎臓を創る　次世代バイオ・医療技術研究会　2015 年 3 月 23 日　東京　（口演）

65. 西中村隆一　iPS 細胞からの腎臓組織の誘導　第８８回日本薬理学会年会　2015 年 3 月 18 日、名古屋（口演＆シンポジウムオーガナイザー）

66. 西中村隆一　試験管内で腎臓を創る　鹿屋地区内科医会学術講演会　2015 年 3 月 13 日　鹿屋、鹿児島　（特別講演）

67. 西中村隆一　試験管内で糸球体を創る　第１０回弥彦ポドサイトセミナー　2015 年 3 月 7 日、新潟　（特別講演）

68. 西中村隆一　ヒト iPS 細胞からの３次元腎臓組織作成　　シンポジウム「科学者たち

による難病への挑戦」　2015 年 2 月 23 日　東京、（口演）

69. 西中村隆一 発生学に基づいた３次元腎臓組織の試験管内構築 京大病院 iPS 細胞・再生医学研究会　2015 年 1 月 30 日、京都　（特別講演）

70. Nishinakamura R. Creating the kidney in vitro. IRCMS Kickoff Symposium, Jan 15, 2015, Kumamoto, Japan. （口演）

71. 賀来祐介、太口敦博、佐久間哲史、山本卓、西中村隆一　ネフロン前駆細胞特異的レポーター遺伝子を持つ iPS 細胞の樹立　第 37 回日本分子生物学会年会　2014 年 11 月 26 日、横浜　(ポスター発表)

72. 谷川俊祐、Sharma N, Yamaguchi T, 西中村隆一、Perantoni A 腎臓ネフロン前駆細胞の初代増幅培養法の確立及び未分化維持機構の解析 第 37 回日本分子生物学会年会 2014 年 11 月 26 日、横浜　(ポスター発表)

73. 田中聡、藤本由佳、山口泰華、立花誠、金井克晃、諸橋憲一郎、川上潔、西中村隆一 転写因子 Six1/Six4 はマウス生殖形成と雄性分化を制御する　第 37 回日本分子生物学会年会　2014 年 11 月 27 日　(口頭発表)

74. 太口敦博、西中村隆一　腎臓構成細胞の起源と多能性幹細胞からの三次元再構築の試み　第 37 回日本分子生物学会年会 2014 年 11 月 26 日、横浜　(口頭発表)

75. Nishinakamura R and Taguchi A. Programming stem cells toward the kidney. 第 37 回日本分子生物学会年会　2014 年 11 月 26 日 横浜 (招待口演)

76. Nishinakamura R. Creating the kidney in vitro. Karolinska Tohoku joint symposium on medical sciences, Nov 8-9, 2014, Sendai, Japan. （口演）

77. 西中村隆一　ヒト iPS 細胞からの３次元腎臓組織作成　CBI (Chem-Bio Infomatics) 学会　大会企画シンポジウム　2014 年 10 月 28 日、東京　（招待講演）

78. 西中村隆一、太口敦博　多能性幹細胞からの３次元腎臓組織の誘導　第 87 回日本生化学会　2014 年 10 月 18 日、京都　（指定講演）

79. 西中村隆一　発生学に基づく３次元腎臓組織の試験管内誘導　第 3 回川島腎カンファレンス　2014 年 10 月 11 日、岐阜　（特別講演）

80. 太口敦博、西中村隆一　新しい腎臓発生モデルの構築とそれに基づく多能性幹細胞から３次元腎臓組織誘導法の確立　第 5 回分子腎臓フォーラム 2014 年 9 月 6 日　東京（口頭発表）

81. Kaku Y and Nishinakamura R. Generation of a GFP reporter human iPS cell line labeling nephron progenitors. 2nd Asia-Pacific Kidney Development Workshop. Sep 22-23, 2014, Queenstown, New Zealand （poster 発表）

82. Tanigawa S, Sharma N, Hall MD, Yamaguchi TP, Nishinakamura R, and Perantoni AO. Maintenance and propagation of rat metanephric mesenchyme cells. 2nd Asia-Pacific Kidney Development Workshop. Sep 22-23, 2014, Queenstown, New Zealand （口頭発表）

83. Taguchi A and Nishinakamura R. A novel model for kidney development and regeneration. 2nd Asia-Pacific Kidney Development Workshop. Sep 22-23, 2014, Queenstown, New Zealand （口頭発表）

84. Nishinakamura R. Transcriptional and morphogenetic regulation of developing nephrons. 2nd Asia-Pacific Kidney Development Workshop. Sep 22-23, 2014, Queenstown, New Zealand （口演）

85. Tanaka SS, Yamaguchi, YL, and Nishinakamura R. Sall4 is essential for mouse primordial germ cell specification by suppressing the somatic cell program. Key Forum: From Stem Cells to Organs, Sep 4, Kumamoto, Japan (ポスター発表)

86. Kaku Y, Taguchi A and Nishinakamura R. Generation of human iPS cell lines labeling nephron progenitors. Key Forum: From Stem Cells to Organs, Sep 4, Kumamoto, Japan (ポスター発表)

87. Tanigawa S, Sharma N, Yamaguchi TP, Nishinakamura R, and Perantoni AO. Development of culture method for

maintenance of rat metanephric mesenchyme progenitor cells. Key Forum: From Stem Cells to Organs, Sep 4, Kumamoto, Japan (ポスター発表)

88. Taguchi A and Nishinakamura R. Redefining the *in vivo* origin of nephron progenitors enables generation of three-dimensional kidney structures *in vitro*. Key Forum: From Stem Cells to Organs, Sep 4, Kumamoto, Japan (実行委員長)

89. 西中村隆一　腎臓の起源同定に基づく３次元腎臓組織の試験管内構築　第 23 回発達腎研究会　2014 年 8 月 31 日、東京　（特別講演）

90. 西中村隆一　腎臓の起源同定に基づく３次元腎臓組織の試験管内構築　臓器再生をめざした再生医療開発の最前線　2014 年 8 月 8 日、品川　（特別講演）

91. 西中村隆一　腎臓の起源同定に基づく３次元腎臓組織の試験管内構築　第 23 回日本小児泌尿器科学会総会　2014 年 7 月 11 日、横浜　（特別講演）

92. 太口敦博、西中村隆一　腎臓の起源の新規同定とそれに基づくヒト iPS 細胞から三次元腎臓組織誘導法の確立　2014 年 7 月 6 日、第 57 回日本腎臓学会　横浜（口頭発表）

93. Tanaka SS, Yamaguchi, YL, Matsui Y, Nishinakamura R. Sall4 is essential for mouse primordial germ cell specification by suppressing the somatic cell program. 第 47 回日本発生生物学会 2014 年 5 月 29 日、名古屋（口頭発表）

94. Tanaka SS, Yamaguchi, YL, Matsui Y, Nishinakamura R. Sall4 is essential for mouse primordial germ cell specification by suppressing the somatic cell program. 第 47 回日本発生生物学会 2014 年 5 月 29 日、名古屋（口頭発表）

95. Taguchi A and Nishinakamura R. Redefining the *in vivo* origin of nephron progenitors enables generation of three-dimensional kidney structures *in vitro*. 腎臓初期発生の新規モデルと多能性幹細胞からの三次元腎臓組織の構築　第 47 回日本発生生物学会 2014 年 5 月 27 日　名古屋（口頭発表）

96. 西中村隆一　Redefining the *in vivo* origin of nephron progenitors enables generation of three-dimensional kidney structures *in vitro*.第 57 回日本糖尿病学会　2014 年　5 月 24 日　大阪（口演）

97. 西中村隆一　腎臓の起源同定に基づく３次元腎臓組織の再構築　第４回徳島腎臓病を考える会　2014 年 5 月 20 日　徳島（特別講演）

98. 西中村隆一　腎臓の起源同定に基づく３次元腎臓組織の再構築　徳島大学藤井節郎記念医科学センター開設記念シンポジウム　2014 年 4 月 10 日　徳島　（特別講演）

99. Taguchi A and Nishinakamura R. Redefining the *in vivo* origin of nephron progenitors enables generation of three-dimensional glomeruli and renal tubules from pluripotent stem cells *in vitro*. The 12th annual meeting of International Society of Stem Cell Research. June 19, 2014, Vancouver, Canada（口頭発表）

100. Nishinakamura R and Taguchi A. Redefining the *in vivo* origin of nephron progenitors enables generation of three-dimensional glomeruli and renal tubules from pluripotent stem cells *in vitro*. The 14th Asian Pacific Congress of Nephrology. May 16, 2014, Tokyo, Japan（口演）

101. Taguchi A and Nishinakamura R. Redefining the *in vivo* Developmental Process of Nephron Progenitors Enables Generation of Three-dimensional Kidney Structures from Pluripotent Stem Cells *in vitro*. The 14th Asian Pacific Congress of Nephrology. May 15, 2014, Tokyo, Japan（口頭発表）

102. 西中村隆一、太口敦博　腎臓の起源同定に基づく幹細胞からの腎臓誘導法の開発　第 119 回日本解剖学会総会シンポジウム 2014 年 3 月 29 日 栃木　（口演）

103. Taguchi A and Nishinakamura R. Redefining the *in vivo* Developmental Process of Nephron Progenitors Enables Generation of Three-dimensional Kidney Structures from Pluripotent Stem Cells *in vitro*. CDB symposium 2014, Mar 12, 2014, Kobe, Japan（口演）.

104. 太口敦博、西中村隆一　腎臓の起源の新規同定とそれに基づく三次元腎臓組織誘導法の確立 第 13 回日本再生医療学会総会 2014 年 3 月 5 日　京都（口演）

105. 西中村隆一、太口敦博　腎臓の起源同定に基づく 3 次元腎臓組織の試験管内構築 熊本ライフサイエンスフォーラム　2014 年 2 月 7 日　熊本　（指定講演）

106. 西中村隆一　発生学と幹細胞学の融合による腎臓再生　第 15 回神田川腎セミナー（招待講演）2014 年 1 月 24 日、東京

107. 田中聡、藤本由佳、山口泰華、立花誠、金井克晃、諸橋憲一郎、川上潔、西中村隆一 転写因子 Six1/Six4 はマウス生殖腺形成と雄性分化を制御する　第 36 回日本分子生物学会年会　2013 年 12 月 5 日、神戸

108. 谷川俊祐、Sharma N, Yamaguchi T, 西中村隆一、Perantoni A. 腎臓ネフロン前駆細胞の未分化維持機構解析及び初代増幅培養法の確立　第 36 回日本分子生物学会年会 2013 年 12 月 5 日、神戸

109. 賀来祐介、大森智子、西中村隆一　Islet1 の欠失は腎臓の形成不全と水尿管症を引き起こす　第 36 回日本分子生物学会年会 2013 年 12 月 5 日、神戸

110. 西中村　隆一　転写因子 Sall1 はネフロン前駆細胞の維持に必須である（口演）第 36 回日本分子生物学会年会　2013 年 12 月 5 日、神戸

111. Nishinakamura R.　The phosphatase Dullard is essential for nephron maintenance after birth. 12th International Workshop on Developmental Nephrology (口演及び organizing committee member). Jun 24, 2013, Edinburgh, Scotland, UK.

112. 西中村隆一、小林俊寛、臼井丈一、渡邊将人、長嶋比呂志、中内啓光　遺伝子改変マウスを用いた腎臓再構築の試み　　器官臓器再生　第 12 回日本再生医療学会 2013 年 3 月 22 日、横浜　（口頭発表及びシンポジウムオーガナイザー）

113. 西中村隆一　ネフロン前駆細胞による腎臓発生機構　熊本和光ライフサイエンスフォーラム　2013 年 2 月 22 日　熊本　（指定講演）

114. Recuenco MC, Ohmori T, Fujimura S, Conti MA, Wei Q, Adelstein RS, Nishinakamura R. Nonmuscle myosin II is essential for nephron development in the embryonic kidney. 日本分子生物学会　2012 年 12 月 14 日、福岡

115. 藤本 由佳, 田中 聡, 山口 泰華, 金井 克晃, 川上 潔, 西中村 隆一 Six1 遺伝子と Six4 遺伝子はマウス生殖腺の雄性分化に必須である 日本分子生物学会　2012 年 12 月 13 日、福岡（口頭発表）

116. Tanaka SS, Yamaguchi YL, Asashima M, Tam PPL and Nishinakamura R.　Dullard/Ctdnep1 modulates WNT signaling activity for the formation of primordial germ cells in the mouse embryo. 日本分子生物学会　2012 年 12 月 12 日、福岡

117. 神田 祥一郎, 西中村 隆一 The role of Sall1 in the kidney development. 日本分子生物学会　2012 年 12 月 11 日、福岡

118. 西中村隆一 Islet1 の欠失による腎臓欠損と水腎症　器官形成− 3 次元構造の構築 H24 日本分子生物学会　2012 年 12 月 12 日、福岡　（口頭発表及びワークショップオーガナイザー）

119. 西中村隆一　腎発生と生殖器　第 16 回心血管内分泌代謝学会　21012 年 11 月 24 日、東京（招待講演）

120. 西中村隆一　ネフロン前駆細胞による腎臓発生と再生　第 7 回代謝異常と CKD を考える会　2012 年 10 月 22 日、仙台（特別講演）

121. 西中村隆一 Nephron progenitors in the embryonic kidney　第 18 回日本遺伝子治療学会　2012 年 6 月 29 日　熊本（ホテルテルサ）　（口演）

122. 西中村隆一 ネフロン前駆細胞による腎臓発生と再生　　小児腎臓病漢方研究会 2012 年 6 月 28 日　東京　（都市タワーホテル）（特別講演）

123. Taguchi A and Nishinakamura R. Identification of early stage renal progenitors in E9.5 embryos by using Osr1-GFP knock-in mice. 10th International Society of Stem Cell Research. Jun 15, 2012, Yokohama, Japan.

124. Kanda S and Nishinakamura R. Sall1 has dual functions in the kidney development. 10th International Society of Stem Cell Research. Jun 14, 2012, Yokohama, Japan.

125. 西中村隆一　腎臓を創る-乗り越えるべき課題と方策　（口演及びシンポジウムオーガナイザー）第55回日本腎臓学会　2012年6月１日　横浜

126. Fujimoto Y, Tanaka SS, Yamauchi YL, Kanai Y, Morohashi K, Kawakami K and Nishinakamura R. Six1 and Six4 homeoproteins are required for sex determination in mouse gonad. The Japanese Society of Developmental Biologists, May 30, 2012, Kobe, Japan.

127. Tanaka SS, Yamauchi YL, Fujimoto Y, Kawakami K and Nishinakamura R. Six1 and Six4 homeodomain proteins act downstream to BMP signal in mouse primordial germ cell formation. The Japanese Society of Developmental Biologists, May 31, 2012, Kobe, Japan.

アウトリーチ活動　Outreach activities

1. JST スーパーサイエンスハイスクール指定校　熊本県立宇土高校　ロジックプログラム講師　谷川俊祐　(日本分子生物学会中学高校への講師派遣事業). 10月13日、2017, 熊本

2. 共同利用・共同研究拠点　知の拠点セミナー　西中村隆一　試験管の中で腎臓を創る　　9月15日、2017,　東京

3. 国立大学附置研究所・センター長会議第２部会 公開講義 西中村隆一「腎臓をつくる」10 月31日、2015年, 熊本

4. 文部科学省の競争的研究費改革に関する検討会　西中村隆一　「熊本大学発生医学研究所の共通支援システムについて」3月13日, 2015年　東京。　その内容が科学新聞の１面に掲載された　3 月20日、2015年

5. 熊本医療都市ネットワーク医療講演会 西中村隆一「ヒト iPS 細胞を用いた再生医学への展望」1月26日2013年、熊本

授賞　Award

1. 谷川俊祐、熊本大学研究業績表彰、ネフロン前駆細胞の試験管内増幅　2017

2. 谷川俊祐、優秀賞、第７回分子腎臓フォーラム、ネフロン前駆細胞の試験管内増幅　2016

3. 田中聡、熊本医学会奨励賞、性決定機構の解明　2015

4. 太口敦博、井上研究奨励賞、腎臓誘導法の開発　2014

5. 太口敦博、熊本大学研究業績表彰、腎臓細胞誘導法の開発　2014

6. 太口敦博、優秀賞、第５回分子腎臓フォーラム、腎臓細胞誘導法の開発　2014

7. 太口敦博、会長賞、日本腎臓学会、腎臓細胞誘導法の開発　2014

8. 西中村隆一、熊本大学教育活動表彰、2014

9. 藤本由佳、熊本大学大学院優秀学生表彰、性決定機構の解明　2013

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ORIGINAL ARTICLE

WILEY Genes to Cells

Dullard deficiency causes hemorrhage in the adult ovarian follicles

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In mammals, the ovarian follicles are regulated at least in part by bone morphogenetic protein (BMP) family members. *Dullard* (also known as *Ctdnep1*) gene encodes a phosphatase that suppresses BMP signaling by inactivating or degrading BMP receptors. Here we report that the *Col1a1-Cre*-induced *Dullard* mutant mice displayed hemorrhagic ovarian cysts, with red blood cells accumulated in the follicles, resulting in infertility. Cells expressing *Cre* driven by *Col1a1* 2.3-kb promoter and their descendants were found in granulosa cells in the ovary and in Sertoli cells in the testis. *Dullard* mRNA was localized to granulosa cells in the ovary. Genes involved in steroid hormone genesis including *Cyp11a1*, *Hsd3b1* and *Star* were reduced, whereas expression of *Smad6* and *Smad7*, BMP-inducible inhibitory Smads, was up-regulated in the *Dullard* mutant ovaries. Tamoxifen-inducible *Dullard* deletion in the whole body using *Rosa26-CreER* mice also resulted in hemorrhagic ovarian cysts in 2 weeks, which was rescued by administration of LDN-193189, a chemical inhibitor of BMP receptor kinase, suggesting that the hemorrhage in the *Dullard*-deficient ovarian follicles might be caused by increased BMP signaling. Thus, we conclude that *Dullard* is essential for ovarian homeostasis at least in part via suppression of BMP signaling.

1 | INTRODUCTION

The human ovary contains approximately 1–2 million primary oocytes at birth and continues to produce eggs through ovulation approximately every 28 days until menopause. This life cycle of ovarian follicles is highly regulated by various hormones including pituitary gonadotrophin hormones (follicle-stimulating hormone [FSH] and luteinizing hormone), gonadotropin-releasing hormone and gonadal hormones (estrogen, androgen and progesterone). However, ovarian follicle

The Era of Human Developmental Nephrology

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In this issue of the *Journal of the American Society of Nephrology*, Lindström *et al.*[1–3] published three milestone papers on human embryonic kidney development. Our current understanding of human kidney development as well as the textbooks of embryology used for learning at medical schools are mostly on the basis of classic examinations of histologic sections performed 50–100 years ago. However, developmental biology has made significant advances since then owing to rapid progress in techniques for molecular biology, imaging, and generation of genetically engineered animals. We now know that the kidney is derived from at least three precursor populations: nephron progenitors that give rise to glomeruli and renal tubules, the ureteric bud that forms the collecting ducts and ureter, and stromal progenitors that produce interstitial cells. Signature genes of these precursor populations as well as markers for their descendant nephron segments have been identified. Many of these genes have been knocked out in mice, and their functions have been studied in detail. Moreover, various mouse strains expressing Cre recombinase or fluorescent reporters have been generated, and the gene expression profiles of many kidney cell lineages have been elucidated by microarray and RNA-sequencing analyses. Despite these advances in mice, little information is available on human kidney development.

In the milestone studies in this issue, Lindström *et al.*[1–3] applied modern technologies, including three-dimensional imaging and RNA sequencing, to the human embryonic kidney. They collected as many as 135 human kidneys ranging from 4 to 23 weeks of gestation (counting from fertilization) and provide a modernized framework for human kidney development. According to their reports, the ureteric bud invades into the metanephric mesenchyme, which contains nephron progenitors, at approximately 4 weeks after fertilization (Carnegie stage 13 [CS13]) compared with embryonic day 11.5 in mice. S-shaped bodies, representing the transition state to nascent glomeruli, are first observed at 6–7 weeks (CS18–CS19), and the kidney structures from 8 weeks (CS23) to 16 weeks are somewhat similar to those in mice at embryonic day 15.5, although the human kidney has multiple lobes. There are many conserved features between the two species, including the overall structures, distinct cell lineage markers, and nascent nephron patterning. However, species-specific differences also exist. For example, some of the marker genes for nephron segments in mice are differentially expressed in humans. Furthermore, human nephron progenitor–specific genes are identified.[2] Meanwhile, Foxd1, the most representative marker for stromal progenitors in mice, is expressed equally in stromal and nephron progenitors in humans, and thus, we may need to reconsider the molecular differences between these two precursor populations. Some of the most informative data for developmental nephrologists are provided by single-cell RNA sequencing of the cortical nephrogenic zone in the 16-week human kidney.[2] On the basis of the gene expression patterns, 2750 cells are classified into multiple clusters representing nephron progenitors, ureteric bud, interstitial cells, vasculature cells, and many others. The nephron progenitors are separated into three subpopulations (naïve, primed, and differentiating cells), and the gene profiles in these subpopulations are elucidated. Although not studied in detail in these reports, other lineages, such as interstitial cells, are also separated into multiple fractions, and further analyses could reveal heterogeneity within these lineages. Examinations at different time points will further accelerate our understanding of the maturation process of the human embryonic kidney.

The above data will serve as useful references for *in vitro* kidney organogenesis. Several groups, including ours, have shown the generation of kidney tissues from human induced pluripotent stem cells (iPSCs).[4–6] These tissues reflect significant advances, and it is exciting to see human glomeruli and renal tubules in a dish. However, it remains unknown which stage of the *in vivo* kidney the organoids correspond to and how similar they are. The culture periods required for kidney induction from human iPSCs range from 16 to 22 days depending on the protocols used. If we assume that human iPSCs represent 2-week embryos on the basis of a transcriptome analysis of monkey embryonic stem cells[7] and if iPSCs follow the same developmental clock as that observed *in vivo*, it would take 2 weeks to form nephron progenitors (CS13) and 2 more weeks to give rise to nascent glomeruli (CS18–CS19). Thus, the *in vitro* nephrogenesis is accelerated or the *in vitro* nephrons are more immature than expected. Lindström *et al.*[1] report that the *Hox11* expression observed in human embryonic kidneys *in vivo* is higher than that previously detected in kidney organoids *in vitro*[5] and propose the importance of

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Article

Higher-Order Kidney Organogenesis from Pluripotent Stem Cells

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SUMMARY

Organogenesis generates higher-order structures containing functional subunits, connective components, and progenitor niches. Despite recent advances in organoid-based modeling of tissue development, recapitulating these complex configurations from pluripotent stem cells (PSCs) has remained challenging. In this study, we report assembly of kidney organoids that recapitulate embryonic branching morphogenesis. By studying the distinct origins and developmental processes of the ureteric bud, which contains epithelial kidney progenitors that undergo branching morphogenesis and thereby plays a central role in orchestrating organ geometry, and neighboring mesenchymal nephron progenitors, we established a protocol for differential induction of each lineage from mouse and human PSCs. Importantly, reassembled organoids developed the inherent architectures of the embryonic kidney, including the peripheral progenitor niche and internally differentiated nephrons that were interconnected by a ramified ureteric epithelium. This selective induction and reassembly strategy will be a powerful approach to recapitulate organotypic architecture in PSC-derived organoids.

INTRODUCTION

Recent progress in biology has enabled the induction of various types of functional organ subunits from pluripotent stem cells (PSCs). In particular, strategies employing the cellular "self-organization" phenomenon have enabled successful generation of three-dimensional (3D) "organoids" in a dish (Lancaster and Knoblich, 2014; Sasai, 2013). However, most of the currently available organoids lack module-module connections and a progenitor niche, namely, the "higher-order structure" of the embryonic organ essential for development of the systemic organ anatomy and functions. Thus, we focused on innate branching morphogenesis by epithelial tissue, which plays a critical role in orchestrating organ geometries (Ochoa-Espinosa and Affolter, 2012).

A rudiment of the kidney, the embryonic metanephros, develops by mutual interaction of the metanephric mesenchyme (MM; including nephron progenitors [NPs] and stromal progenitors [SPs]) and the ureteric bud (UB) (Costantini and Kopan, 2010). The UB undergoes branching morphogenesis to form urine-collecting systems, and the tips of the UB signal to maintain undifferentiated NPs and induce differentiation of a subset of NPs. In this process, a transient Wnt signal from the UB induces mesenchymal-to-epithelial transition (MET) of NPs, and each epithelialized nephron then attaches to the UB tips for connection to the collecting duct. In turn, the undifferentiated NPs produce Gdnf to maintain UB tip proliferation, and the surrounding cortical SPs support ureteric branching by maintaining Ret receptor tyrosine kinase expression in the UB tips. This triad interaction enables concomitant NP maintenance and differentiation, thereby producing millions of nephrons with systemic connections. Hence, the roles of the UB, including dichotomous branch formation, NP maintenance, and NP differentiation, are essential for organ-scale kidney morphogenesis.

Recently, several groups have reported induction of the renal lineage from PSCs. We and another group demonstrated selective induction of the NP lineage (Taguchi et al., 2014; Morizane et al., 2015). Other groups have shown the derivation of a UB-like population by selective (Xia et al., 2013) or simultaneous (Takasato et al., 2015) induction with NP and SP populations. Most protocols that aimed to include the NP lineage resulted in epithelial nephron-like structure formation to a certain extent (Taguchi et al., 2014; Morizane et al., 2015; Takasato et al., 2015). However, the induced UB-like cells did not show branching morphogenesis and the NP induction/maintenance capacity was not proved, and therefore the inter-nephron connection by the collecting ducts was lacking (Xia et al., 2013; Takasato et al., 2015). These findings suggest that the currently available UB induction protocols are not sufficient to induce a functional UB, which could be partly due to the lack of precise knowledge about the differentiation signals for the early-stage UB lineage (Costantini and Kopan, 2010).

Previously, we identified spatiotemporally distinct origins of the UB and the MM (NP+SP) (Taguchi et al., 2014; Taguchi and Nishinakamura, 2015). The UB differentiates from the T^+ immature mesoderm at embryonic day (E) 7.5. Subsequently, at E8.5, the immature mesoderm becomes the $Osr1^+/Lhx1^+/Pax2^+/T^-$ anterior intermediate mesoderm (AIM). The anteriorly located committed UB lineage precursors extend and migrate caudally to form an elongated epithelial tube, the Wolffian

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RESEARCH ARTICLE

Kinesin superfamily protein Kif26b links Wnt5a-Ror signaling to the control of cell and tissue behaviors in vertebrates

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Abstract Wnt5a-Ror signaling constitutes a developmental pathway crucial for embryonic tissue morphogenesis, reproduction and adult tissue regeneration, yet the molecular mechanisms by which the Wnt5a-Ror pathway mediates these processes are largely unknown. Using a proteomic screen, we identify the kinesin superfamily protein Kif26b as a downstream target of the Wnt5a-Ror pathway. Wnt5a-Ror, through a process independent of the canonical Wnt/β-catenin-dependent pathway, regulates the cellular stability of Kif26b by inducing its degradation via the ubiquitin-proteasome system. Through this mechanism, Kif26b modulates the migratory behavior of cultured mesenchymal cells in a Wnt5a-dependent manner. Genetic perturbation of Kif26b function in vivo caused embryonic axis malformations and depletion of primordial germ cells in the developing gonad, two phenotypes characteristic of disrupted Wnt5a-Ror signaling. These findings indicate that Kif26b links Wnt5a-Ror signaling to the control of morphogenetic cell and tissue behaviors in vertebrates and reveal a new role for regulated proteolysis in noncanonical Wnt5a-Ror signal transduction.
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Introduction

The Wnt family of extracellular signaling factors orchestrates diverse developmental processes during both embryogenesis and adult tissue homeostasis. Dysfunction of Wnt signaling has been implicated in many human diseases ranging from congenital birth defects to neoplasia (***Clevers and Nusse, 2012***; ***Kikuchi et al., 2012***). Wnt ligands achieve high functional versatility in part by

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Re-expression of *Sall1* in podocytes protects against adriamycin-induced nephrosis

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The highly conserved spalt (sal) gene family members encode proteins characterized by multiple double zinc finger motifs of the C2H2 type. Humans and mice each have four known Sal-like genes (*SALL1–4* in humans and *Sall1–4* in mice). *Sall1* is known to have a crucial role in kidney development. To explore the significance of *Sall1* in differentiated podocytes, we investigated podocyte-specific *Sall1*-deficient mice (*Sall1* KO$^{pod/pod}$) using a podocin-Cre/loxP system and siRNA *Sall1* knockdown (*Sall1* KD) podocytes. Under physiological conditions, *Sall1* KO$^{pod/pod}$ mice exhibited no proteinuria during their lifetime, but foot-process effacement was detected in some of the podocytes. To elucidate the role of *Sall1* in injured podocytes, we used an adriamycin (ADR)-induced model of nephrosis and glomerulosclerosis. Surprisingly, the expression of *Sall1* was elevated in control mice on day 14 after ADR injection. On day 28 after ADR injection, *Sall1* KO$^{pod/pod}$ mice exhibited significantly higher levels of proteinuria and higher numbers of sclerotic glomeruli. Differentiated *Sall1* KD podocytes showed a loss of synaptopodin, suppressed stress fiber formation, and, ultimately, impaired directed cell migration. In addition, the loss of *Sall1* increased the number of apoptotic podocytes following ADR treatment. These results indicated that *Sall1* has a protective role in podocytes; thus, we investigated the endoplasmic reticulum stress marker GRP78. GRP78 expression was higher in ADR-treated *Sall1* KO$^{pod/pod}$ mice than in control mice. *Sall1* appeared to influence the expression of GRP78 in injured podocytes. These results suggest that *Sall1* is associated with actin reorganization, endoplasmic reticulum stress, and apoptosis in injured podocytes. These protective aspects of *Sall1* re-expression in injured podocytes may have the potential to reduce apoptosis and possibly glomerulosclerosis.

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Podocyte foot processes (FP) and their interposed slit diaphragms (SD) are key components of the permeability barrier in glomeruli. Podocyte damage or loss can severely impair kidney function and is an early symptom of many kidney diseases involving nephrotic syndrome and/or glomerulosclerosis. Podocyte FP effacement and/or molecular reorganization of the SD are characteristic pathological features of nephrotic syndrome.[1] Elucidating the molecular mechanisms involved in the response of podocytes to damage is essential to understand podocyte pathogenesis.

The Spalt (sal) gene family encodes zinc finger proteins that both control normal development and apparently function as tumor suppressors in humans and mice.[2] *SALL1–SALL4* in humans have DNA sequence homologies with the Drosophila *sal* gene.[3] The Sall1 protein binds to A/T-rich sequences of the major satellite DNA via its C-terminal double zinc fingers, thereby localizing it to heterochromatin.[4] This protein also functions as a transcriptional repressor.[5] In humans, *SALL1* mutations cause an autosomal dominant disorder characterized by limb, ear, anus, heart, and kidney malformations.[6]

The importance of *Sall1* in kidney development has been investigated using *Sall1* knockout mice. Homozygous *Sall1* knockout mice die from kidney agenesis or severe dysgenesis within 24 h after birth.[7] *Sall1* is essential for ureteric bud

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SCIENTIFIC REPORTS

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PAX2 is dispensable for *in vitro* nephron formation from human induced pluripotent stem cells

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The kidney is formed by reciprocal interactions between the nephron progenitor and the ureteric bud, the former of which gives rise to the epithelia of nephrons consisting of glomeruli and renal tubules. The transcription factor *PAX2* is essential for this mesenchymal-to-epithelial transition of nephron progenitors, as well as ureteric bud lineage development, in mice. *PAX2* mutations in humans cause renal coloboma syndrome. We previously reported the induction of nephron progenitors and three-dimensional nephron structures from human induced pluripotent stem (iPS) cells. Here we generate iPS cells lacking *PAX2*, and address the role of *PAX2* in our *in vitro* induction protocol. While *PAX2*-null human nephron progenitors were properly formed, they unexpectedly became epithelialised to form glomeruli and renal tubules. However, the mutant glomerular parietal epithelial cells failed to transit to the squamous morphology, retaining the shape and markers of columnar epithelia. Therefore, *PAX2* is dispensable for mesenchymal-to-epithelial transition of nephron progenitors, but is required for morphological development of glomerular parietal epithelial cells, during nephron formation from human iPS cells *in vitro*.

The mammalian kidney, the metanephros, is formed by reciprocally inductive interactions between two precursor tissues, the metanephric mesenchyme and the ureteric bud[1]. The metanephric mesenchyme gives rise to glomeruli and renal tubules, while the ureteric bud branches to create collecting ducts and ureters, thus forming the nephron, a functional unit of the kidney. The metanephric mesenchyme contains nephron progenitors that undergo mesenchymal-to-epithelial transition upon induction by the ureteric bud. The progenitors sequentially transit to renal vesicles, C-shaped bodies, and S-shaped bodies, eventually forming glomeruli and renal tubules. The proximal region of S-shaped bodies becomes the glomeruli and proximal renal tubules, while the distal region becomes the distal renal tubules. The glomerular epithelia are further segregated into two lineages: visceral epithelial cells (podocytes) and parietal epithelial cells (Bowman's capsule epithelial cells). Podocytes exhibit a unique morphology, having multiple cellular processes bridged by slit diaphragms, which are filtration apparatuses consisting of transmembrane proteins including NEPHRIN[2]. In contrast, glomerular parietal cells become flattened and adopt a squamous shape. Urine, which is filtrated through the slit diaphragms of the podocytes, flows into the space surrounded by the glomerular parietal epithelial cells (Bowman's capsule), and then into the adjacent proximal renal tubules, distal renal tubules, collecting ducts, and ureters.

There is an *ex vivo* culture system available to assess the competence of the metanephric mesenchyme. In the system, isolated metanephric mesenchyme is co-cultured with embryonic spinal cord, and the nephron progenitors in the mesenchyme undergo mesenchymal-to-epithelial transition to form nephron structures, including glomeruli and renal tubules. In this setting, the spinal cord functions as a substitute for the ureteric bud, as both can secrete Wnt ligands and induce differentiation of nephron progenitors[3]. Many mutant mice have been analysed using this spinal cord recombination system[4–6].

Paired box (PAX) genes are homologues of the *Drosophila* pair rule gene *paired* and encode nuclear proteins characterised by DNA-binding paired box domains[7,8]. The *PAX* family has nine members in mammals, and is categorised into four paralogue groups. *PAX2* constitutes one of the groups with *PAX5* and *PAX8*, and plays important roles in organ development. In the developing mouse kidney, Pax2 is expressed in the nephron progenitors,

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MEETING REVIEW

Human development, heredity and evolution

Ryuichi Nishinakamura[1,*] and Minoru Takasato[2]

ABSTRACT

From March 27-29 2017, the RIKEN Center for Developmental Biology held a symposium entitled 'Towards Understanding Human Development, Heredity, and Evolution' in Kobe, Japan. Recent advances in technologies including stem cell culture, live imaging, single-cell approaches, next-generation sequencing and genome editing have led to an expansion in our knowledge of human development. Organized by Yoshiya Kawaguchi, Mitinori Saitou, Mototsugu Eiraku, Tomoya Kitajima, Fumio Matsuzaki, Takashi Tsuji and Edith Heard, the symposium covered a broad range of topics including human germline development, epigenetics, organogenesis and evolution. This Meeting Review provides a summary of this timely and exciting symposium, which has convinced us that we are moving into the era of science targeted on humans.

KEY WORDS: Human development, Single-cell sequencing, Epigenetics, Organoid, Evolution

Introduction

Developmental biology research has a long history of studying model organisms such as the fruit fly *Drosophila melanogaster*, focusing mainly on early embryogenesis. One major revolution in the field came in the early 1990s, when knockout mouse technology led to a focus on embryogenesis and organ development in mammals. Now, recent advances in human embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC) technology have enabled the generation of human organoids (miniature versions of human tissues and organs grown in culture) of a variety of tissues, which can at least partially mimic human diseases and potentially be used in drug discovery platforms. Genome editing is further accelerating these research activities. In addition, single-cell sequencing is becoming a powerful tool for developmental biology, revealing the states of individual cells during embryogenesis and hence allowing investigation of the dynamics of tissue and organ development in greater detail. This technology is now being applied to human embryos, as well organoids generated *in vitro*. Human embryos are obtained and analyzed routinely at some institutions to study human development, not only for RNA sequencing but also for live imaging. These technological advances made for a very timely symposium, although the scope of this meeting was in fact much broader than our expectations. Rapid progress in next-generation sequencing has enabled comparative studies between humans and other mammals that are revealing the evolutionary processes that led to modern humans. Thus, we are now in the era of human developmental biology. In this review of the recent RIKEN Center for Developmental Biology (CDB) symposium 'Towards Understanding Human Development, Heredity, and Evolution' (Fig. 1), we summarize some of the prominent talks and take-home messages from the symposium, beginning with the investigation of early human embryogenesis and germline development, before considering talks focused on the epigenetic regulation of development, organogenesis and, finally, heredity and evolution of the human lineage.

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Germline development and early embryogenesis

Mitinori Saitou (Kyoto University, Japan) previously succeeded in inducing mouse ESCs (mESCs) toward primordial germ cell (PGC) fate, generating cells that could contribute to spermatogenesis and oogenesis and to fertile offspring (Hayashi et al., 2011, 2012; Nakaki et al., 2013). He also reported PGC-like cells from human iPSCs (hiPSCs) (Sasaki et al., 2015). In his presentation, he discussed single-cell sequencing of pre- and post-implantation embryos of cynomolgus monkey, setting up a reference of normal early embryogenesis in non-human primates. This revealed that monkey ESCs and hiPSCs are transcriptionally similar to post-implantation late stage epiblasts, whereas mESCs correspond to the pre-implantation epiblasts (Nakamura et al., 2016). He further showed that primate PGCs do not appear in the epiblast but rather in the amnion as $SOX17^{+}$ $TFAP2C^{+}$ $BLIMP1^{+}$ cells and then migrate toward the epiblast (Sasaki et al., 2016). The inductive roles of Wnt and BMP in PGC specification are conserved from mouse, but their source is different: Wnt is provided from cytotrophoblast and BMP4 from amnion. This illustrates important species-specific differences and highlights the limitations of the mouse as a model of human development. Reconciling this finding – that human PGCs (hPGCs) first appear in the amnion – with the fact that hPGCs can be induced from hiPSCs through an incipient mesoderm-like state will require further investigation. Azim Surani (University of Cambridge, UK) also reported that the mechanisms of PGC specification between human and mice are not conserved. He showed that, unlike in mouse where SOX2, BLIMP1 (PRDM1) and PRDM14 are required for PGC specification, SOX17 and BLIMP1 could induce hPGCs from ESCs *in vitro*. Equal dosage of these two factors is important for hPGC specification; dominant expression of SOX17 over BLIMP1 produces aberrant cells with expression of some definitive endoderm genes. Furthermore, Surani obtained human embryos to investigate the *in vivo* development of hPGCs. In these samples, human-specific 'escapee' loci that evade germline reprogramming could be identified, revealing potential for transgenerational epigenetic inheritance that may have phenotypic consequences (Tang et al., 2015).

Patrick Chinnery (University of Cambridge, UK) studies mitochondrial DNA (mtDNA) heteroplasmy within the germline. Heteroplasmic mtDNA mutations cause inherited metabolic diseases when the proportion of mutant mitochondria exceeds a critical threshold. Interestingly, the phenotype varies widely even within the same family depending on the level of mtDNA heteroplasmy. Such variation between individuals can be explained by the 'bottleneck theory', in which the number of

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Original research article

Non-muscle myosin II deletion in the developing kidney causes ureter-bladder misconnection and apical extrusion of the nephric duct lineage epithelia

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ABSTRACT

In kidney development, connection of the nephric duct (ND) to the cloaca and subsequent sprouting of the ureteric bud (UB) from the ND are important for urinary exit tract formation. Although the roles of Ret signaling are well established, it remains unclear how intracellular cytoskeletal proteins regulate these morphogenetic processes. *Myh9* and *Myh10* encode two different non-muscle myosin II heavy chains, and *Myh9* mutations in humans are implicated in congenital kidney diseases. Here we report that ND/UB lineage-specific deletion of *Myh9/Myh10* in mice caused severe hydroureter/hydronephrosis at birth. At mid-gestation, the mutant ND/UB epithelia exhibited aberrant basal protrusion and ectopic UB formation, which likely led to misconnection of the ureter to the bladder. In addition, the mutant epithelia exhibited apical extrusion followed by massive apoptosis in the lumen, which could be explained by reduced apical constriction and intercellular adhesion mediated by E-cadherin. These phenotypes were not ameliorated by genetic reduction of the tyrosine kinase receptor *Ret*. In contrast, ERK was activated in the mutant cells and its chemical inhibition partially ameliorated the phenotypes. Thus, myosin II is essential for maintaining the apicobasal integrity of the developing kidney epithelia independently of Ret signaling.

1. Introduction

The kidney develops by reciprocal induction between two precursor tissues: the metanephric mesenchyme and the ureteric bud (UB). The former gives rise to the upper part of the nephron (glomeruli and renal tubules), while the latter contributes to the lower part of the nephron and urinary exit tract (collecting ducts and ureters). At embryonic day (E) 9.5 in the mouse, the nephric duct (ND; Wolffian duct) elongates and reaches the cloaca. At E10.5–11.5, the UB sprouts from the ND and invades the metanephric mesenchyme, thereby inducing the mesenchyme to transform into the nephron epithelia. Simultaneously, the UB branches extensively and forms a tree-like structure consisting of collecting ducts and ureters. The initial sprouting site of the UB and its branching patterns are strictly controlled, and its main regulator is glial cell line-derived neurotrophic factor (GDNF)-Ret signaling (Costantini and Kopan, 2010). GDNF secreted from the metanephric mesenchyme acts on the Ret tyrosine kinase receptor on the ND/UB epithelia. Ret signaling leads to activation of a phosphorylation cascade including ERK and stimulates many downstream target genes, such as *Etv4/5*, *Wnt11*, and *Ret* itself (Lu et al., 2009). Thus, *Ret* or *Gdnf* deficiency results in reduced UB branching and eventually kidney agenesis (Costantini and Kopan, 2010; Durbec et al., 1996). In contrast, deletion of negative regulators, such as *Robo2* and *Spry1* (Grieshammer et al., 2004; Basson et al., 2005), and a hyperactive *Ret* mutant (Hoshi et al., 2012) lead to excessive ERK activation, positional shift of the UB sprouting site, and ectopic UB budding. The position of the UB sprouting site is critical for the subsequent proper connection between the ureter and the bladder. In normal development, the ND region caudal to the UB sprouting site, which is called the common nephric duct (CND), undergoes physiological apoptosis, and the distal end of the UB eventually connects to the bladder directly (Mendelsohn, 2009; Stewart and Bouchard, 2014). The above-described disorders leading to excessive *Ret* activation impair this process, leading to misconnection of the ureter and the bladder, and eventually to dilatation of the ureters (hydroureter), and in more severe cases, dilatation of the urinary tract in the kidney (hydronephrosis) (Grieshammer et al., 2004; Basson et al.,

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Chapter 13

Generation of a Three-Dimensional Kidney Structure from Pluripotent Stem Cells

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Abstract

The kidney is a vital organ that has an important role in the maintenance of homeostasis by fluid volume regulation and waste product excretion. This role cannot be performed without the three-dimensional (3D) structure of the kidney. Therefore, it is important to generate the 3D structure of the kidney when inducing functional kidney tissue or the whole organ from pluripotent stem cells. In this chapter, we describe the detailed methods to induce kidney progenitor cells from pluripotent stem cells, which are based on embryological development. We also provide a method to generate 3D kidney tissue with vascularized glomeruli upon transplantation.

Key words Three-dimensional kidney structure, Nephron progenitor cells, Mouse embryonic stem cells, Human-induced pluripotent stem cells, Transplantation

1 Introduction

The kidney contains a large number of nephrons, functional units of the kidney, which consist of glomeruli and renal tubules. The main functions of the kidney, including filtration, reabsorption, and excretion, are performed within the nephrons. Patients with end-stage renal disease have lost these functions and need to be treated with dialysis or kidney transplantation. Research involving stem cell-induced kidneys has the potential to realize new therapies for patients.

The mammalian adult kidney is derived from the embryonic metanephros. It is generated from two different progenitor populations: the metanephric mesenchyme (MM) and ureteric bud (UB) [1]. The progenitor cells of the nephron epithelia exist in the MM and are induced to differentiate into glomerular epithelial cells and renal tubular epithelial cells by Wnt signaling from the UB [2]. Meanwhile, the UB differentiates into the epithelia of the collecting duct and ureter. To create a kidney structure from pluripotent stem

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Cooperative Action between SALL4A and TET Proteins in Stepwise Oxidation of 5-Methylcytosine

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SUMMARY

TET family enzymes successively oxidize 5-methylcytosine to 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine, leading to eventual demethylation. 5hmC and TET enzymes occupy distinct chromatin regions, suggesting unknown mechanisms controlling the fate of 5hmC within diverse chromatin environments. Here, we report that SALL4A preferentially associates with 5hmC in vitro and occupies enhancers in mouse embryonic stem cells in a largely TET1-dependent manner. Although most 5hmC at SALL4A peaks undergoes further oxidation, this process is abrogated upon deletion of *Sall4* gene, with a concomitant reduction of TET2 at these regions. Thus, SALL4A facilitates further oxidation of 5hmC at its binding sites, which requires its 5hmC-binding activity and TET2, supporting a collaborative action between SALL4A and TET proteins in regulating stepwise oxidation of 5mC at enhancers. Our study identifies SALL4A as a 5hmC binder, which facilitates 5hmC oxidation by stabilizing TET2 association, thereby fine-tuning expression profiles of developmental genes in mouse embryonic stem cells.

INTRODUCTION

5-methylcytosine (5mC) is an important epigenetic modification, which functions in gene silencing, imprinting control, and X chromosome inactivation (Bestor et al., 2015; Jaenisch and Bird, 2003; Jones, 2012; Schübeler, 2015; Smith and Meissner, 2013). TET enzymes successively oxidize 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC) (He et al., 2011; Ito et al., 2010, 2011; Tahiliani et al., 2009; Zhang et al., 2010), which may lead to eventual demethylation with the assistance of thymine DNA glycosylase (TDG) and the base excision repair machinery (He et al., 2011; Maiti and Drohat, 2011).

5hmC is more stable and abundant than 5fC and 5caC in the genome (Bachman et al., 2014; Kriaucionis and Heintz, 2009). The persistence of 5hmC in the genome raises a question of whether and how 5hmC exerts its own regulatory functions. One possible mechanism suggests that 5hmC may recruit or expel specific proteins, whose DNA-binding activities are sensitive to the 5-hydroxymethyl group (Hashimoto et al., 2012; Kohli and Zhang, 2013; Song and He, 2013). Several proteins, including UHRF2, were reported to selectively recognize 5hmC in vitro (Iurlaro et al., 2013; Spruijt et al., 2013; Yildirim et al., 2011), and we revealed the structural basis for the selectivity of UHRF2 toward 5hmC (Zhou et al., 2014). However, the roles of 5hmC-binding proteins in the context of 5mC oxidation remain largely unknown.

In embryonic stem cells (ESCs), the oxidized forms of 5mC are enriched at enhancers (Shen et al., 2013; Song et al., 2013; Stroud et al., 2011; Yu et al., 2012), and deletion of *Tet* genes causes an accumulation of 5mC at enhancers (Hon et al., 2014; Lu et al., 2014). Similarly, mutations in the *Tet2* gene lead to DNA hypermethylation of enhancers and deregulation of corresponding genes in acute myeloid leukemia cells, which potentially contributes to myeloid tumorigenesis (Rasmussen et al., 2015).

Notably, only a subset of 5hmC in mouse ESCs is further oxidized, which leads to eventual demethylation, with the assistance of TDG (Lu et al., 2015; Shen et al., 2013; Song et al., 2013; Wu et al., 2014; Xia et al., 2015). Structural analysis of TET enzymes revealed an intrinsic property of TET enzymes in stalling

nature immunology

Sall1 is a transcriptional regulator defining microglia identity and function

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Microglia are the resident macrophages of the central nervous system (CNS). Gene expression profiling has identified *Sall1*, which encodes a transcriptional regulator, as a microglial signature gene. We found that *Sall1* was expressed by microglia but not by other members of the mononuclear phagocyte system or by other CNS-resident cells. Using Sall1 for microglia-specific gene targeting, we found that the cytokine receptor CSF1R was involved in the maintenance of adult microglia and that the receptor for the cytokine TGF-β suppressed activation of microglia. We then used the microglia-specific expression of *Sall1* to inducibly inactivate the murine *Sall1* locus *in vivo*, which resulted in the conversion of microglia from resting tissue macrophages into inflammatory phagocytes, leading to altered neurogenesis and disturbed tissue homeostasis. Collectively, our results show that transcriptional regulation by Sall1 maintains microglial identity and physiological properties in the CNS and allows microglia-specific manipulation *in vivo*.

Microglia are the resident macrophages in the CNS parenchyma. They are self-maintained and are not replaced by circulating precursor cells under physiological conditions[1–3]. Early during embryonic development in the extra-embryonic yolk sac, primitive macrophages take up residence in the brain, where they eventually give rise to microglia in the adult[2,4–7]. The development of microglia is dependent on the macrophage-colony-stimulating factor (M-CSF or CSF1) receptor kinase CSF1R, which can transmit signals through the two ligands, CSF1 and interleukin-34 (IL-34)[2,8]. Reports have shown that the development of microglia also relies on the transcription factors IRF8 and PU.1 and the cytokine transforming growth factor-β (TGF-β)[6,9]. One of the reasons that the understanding of microglia biology is still relatively limited is the fact that conditional gene targeting of microglia often leads to inadvertent targeting of other members of the mononuclear phagocyte system (MPS), including monocytes and macrophages.

As innate immune cells, microglia are often associated with CNS pathologies, yet their roles in the development and progression of neuroinflammatory and neurodegenerative diseases remain poorly understood. Evidence has suggested that microglia are crucial for neuronal development as well as for adult neurogenesis and synapse formation[10,11]. Indeed, it is becoming increasingly clear that tissue macrophages in general are vital for tissue homeostatic functions. This is further reflected by the specific ontogenetic niches and phenotypic specializations of resident macrophages across all tissues. This tissue-specific identity is tightly regulated throughout development and adulthood through the transcriptional regulation of signature genes uniquely expressed by macrophages[12,13]. Several signature genes have been proposed for microglia[9,14–16]. Among these, *Sall1*, which encodes the transcriptional regulator Sall1, has been found to have high expression levels in adult microglia[14,17]. *Sall1* is one of four members of the *Spalt* ('Spalt-like' (Sall)) family of evolutionarily conserved genes that were originally identified in *Drosophila* and are critical for organogenesis[18]. Sall1 is a zinc-finger transcription factor that is expressed during embryogenesis in the CNS, limb buds, heart and kidneys[19–21]. Mice lacking Sall1 are not viable as a result of a severe kidney dysgenesis[21]. In humans, heterozygous mutations of *SALL1* can lead to Townes-Brocks syndrome, an autosomal dominant developmental disorder that is characterized by kidney and heart anomalies that accompany anal, limb and auditory abnormalities[22]. In addition, homozygous mutations in *SALL1* can lead to 'multiple congenital anomaly mental retardation syndrome'[23].

We found that expression of *Sall1* was restricted largely to microglia in the hematopoietic compartment and the adult CNS. Using mice with tamoxifen-inducible expression of Cre recombinase under the control of the *Sall1* promoter ($Sall1^{CreER}$)[24], we efficiently targeted microglia and spared otherwise phenotypically similar members of the MPS. Although CSF1R signaling was indeed critical for the maintenance of adult microglia, specific deletion of the TGF-β receptor (TGF-βR) resulted in rapid conversion of microglia toward an inflammatory macrophage phenotype. Furthermore, we found that Sall1-mediated transcriptional control maintained microglial identity *in vivo*, primarily by silencing an inflammatory program in the otherwise 'immunologically unique' CNS. Collectively, our data identify a transcriptional regulator that connects a defined tissue macrophage with its location-specific functions during CNS tissue homeostasis.

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hemodialysis randomized to continue recombinant erythropoietin. Serum hepcidin levels decreased in response to GSK1278863 treatment in the participants with predialysis CKD, but not in those on long-term hemodialysis.[8]

The most recent phase 2 study is reported by Pergola *et al.*[9] in the current issue. The investigators conducted a 20-week, double-blind, randomized, placebo-controlled phase 2b study to evaluate the efficacy and safety of once-daily vadadustat (AKB-6548) in patients with CKD stages 3a to 5. Randomization was stratified by baseline hemoglobin and ESA use. The primary endpoint was the percentage of participants who achieved or maintained either a mean hemoglobin level ≥11.0 g/dl or a mean increase in hemoglobin ≥1.2 g/dl from baseline during the last 2 weeks of treatment. The primary endpoint was met in 54.9% of patients on vadadustat and 10.3% of patients on placebo. The vadadustat group also had significant decreases in both serum hepcidin and ferritin levels compared with the placebo group.[9]

Overall, the PHD inhibitors were considered to be safe and well tolerated at the doses and durations tested. Taken together, these studies found that PHD inhibitors raised and maintained hemoglobin levels in a predictable and controlled manner. The PHD inhibitors also improved markers of iron metabolism, although they were not powered to detect differences in supplemental iron requirements. These studies provide a strong rationale for ongoing phase 3 studies, designed to have sufficient power and follow-up time to assess hard clinical endpoints and to determine whether PHD inhibitors are a safe and effective alternative for anemia management in patients with CKD.

DISCLOSURE

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REFERENCES

1. Pfeffer MA, Burdmann EA, Chen CY, et al. A trial of darbepoetin alfa in type 2 diabetes and chronic kidney disease. *N Engl J Med*. 2009;361:2019–2032.
2. Singh AK, Szczech L, Tang KL, et al., for the CHOIR Investigators. Correction of anemia with epoetin alfa in chronic kidney disease. *N Engl J Med*. 2006;355:2085–2098.
3. Zumbrennen-Bullough K, Babitt JL. The iron cycle in chronic kidney disease (CKD): from genetics and experimental models to CKD patients. *Nephrol Dial Transplant*. 2014;29:263–273.
4. Maxwell PH, Eckhardt KU. HIF prolyl hydroxylase inhibitors for the treatment of renal anaemia and beyond. *Nat Rev Nephrol*. 2016;12:157–168.
5. Provenzano R, Besarab A, Sun CH, et al. Oral hypoxia-inducible factor prolyl hydroxylase inhibitor roxadustat (FG-4592) for the treatment of anemia in patients with CKD. *Clin J Am Soc Nephrol*. 2016;11:982–991.
6. Provenzano R, Besarab A, Wright S, et al. Roxadustat (FG-4592) versus epoetin alfa for anemia in patients receiving maintenance hemodialysis: a phase 2, randomized, 6- to 19-week, open-label, active-comparator, dose-ranging, safety and exploratory efficacy study. *Am J Kidney Dis*. 2016;67:912–924.
7. Besarab A, Chernyavskaya E, Motylev I, et al. Roxadustat (FG-4592): correction of anemia in incident dialysis patients. *J Am Soc Nephrol*. 2016;27:1225–1233.
8. Holdstock L, Meadowcroft AM, Maier R, et al. Four-week studies of oral hypoxia-inducible factor-prolyl hydroxylase inhibitor GSK1278863 for treatment of anemia. *J Am Soc Nephrol*. 2016;27:1234–1244.
9. Pergola PE, Spinowitz BS, Hartman CS, et al. Vadadustat, a novel oral HIF stabilizer, provides effective anemia treatment in nondialysis-dependent chronic kidney disease. *Kidney Int*. 2016;90:1115–1122.

cell biology

Expanding nephron progenitors *in vitro*: a step toward regenerative medicine in nephrology

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With recent success in directed differentiation of nephron progenitors from mouse embryonic stem cells or human-induced pluripotent stem cells, the ability to expand these nephron progenitors is an important step toward regenerative medicine in nephrology. A recent publication reports the first successful attempt to propagate human nephron progenitors while retaining their potential to form both glomeruli and renal tubules.

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Sall4 controls differentiation of pluripotent cells independently of the Nucleosome Remodelling and Deacetylation (NuRD) complex

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ABSTRACT

Sall4 is an essential transcription factor for early mammalian development and is frequently overexpressed in cancer. Although it is reported to play an important role in embryonic stem cell (ESC) self-renewal, whether it is an essential pluripotency factor has been disputed. Here, we show that Sall4 is dispensable for mouse ESC pluripotency. Sall4 is an enhancer-binding protein that prevents precocious activation of the neural gene expression programme in ESCs but is not required for maintenance of the pluripotency gene regulatory network. Although a proportion of Sall4 protein physically associates with the Nucleosome Remodelling and Deacetylase (NuRD) complex, Sall4 neither recruits NuRD to chromatin nor influences transcription via NuRD; rather, free Sall4 protein regulates transcription independently of NuRD. We propose a model whereby enhancer binding by Sall4 and other pluripotency-associated transcription factors is responsible for maintaining the balance between transcriptional programmes in pluripotent cells.

KEY WORDS: Sall4, NuRD, ES cells, Enhancer, Transcription factor, Co-repressor

INTRODUCTION

Embryonic stem cells (ESCs) have the potential to form any somatic cell type in the adult organism; that is, they are pluripotent. In order to properly execute lineage decisions, pluripotent cells must precisely coordinate their gene expression programmes. To successfully initiate differentiation down one particular lineage, a cell must activate the gene regulatory network (GRN) appropriate to enter that lineage, and not those corresponding to any other lineage, while also extinguishing the pluripotency GRN. It is clear from a large number of studies that the coordinated action of multiple transcription factors and chromatin-modifying proteins is essential to maintain the delicate balance between self-renewal and differentiation of ESCs (Morey et al., 2015; Niwa, 2007; Signolet and Hendrich, 2015). Although it is relatively straightforward to show that a given protein plays some role in ESC differentiation, often the precise mechanisms of how the important transcription factors function remain ill-defined.

In this study we focus on Sall1 and Sall4, the only two members of the *spalt* gene family of C2H2-type zinc-finger transcription factors that are expressed in ESCs (reviewed by de Celis and Barrio, 2009). In humans, mutations in *SALL4* show haploinsufficiency, resulting in the autosomal dominant Okihiro/Duane-Radial Ray and IVIC syndromes (Al-Baradie et al., 2002; Kohlhase et al., 2002; Sweetman and Munsterberg, 2006), while mutations in *SALL1* lead to the autosomal dominant Townes-Brocks syndrome (Kohlhase et al., 1998). *SALL4* is also aberrantly expressed in many cancers and correlates with poor prognosis, leading it to be heralded as a new cancer biomarker and potential therapeutic target (Zhang et al., 2015). In mice, Sall4 has been shown to play an essential role in peri-implantation development (Elling et al., 2006; Sakaki-Yumoto et al., 2006; Warren et al., 2007), while Sall1 is dispensable for early embryogenesis but is essential for kidney development (Kanda et al., 2014; Nishinakamura et al., 2001).

The role played by Sall4 in ESCs has been the subject of some debate. Studies using *Sall4* null ESCs concluded that it was dispensable for self-renewal of ESCs, but that mutant cells were prone to differentiate in certain conditions, indicating that it might function to stabilise the pluripotent state (Sakaki-Yumoto et al., 2006; Tsubooka et al., 2009; Yuri et al., 2009). By contrast, studies in which Sall4 was knocked down in ESCs led to the conclusion that it plays an important role in the maintenance of ESC self-renewal (Rao et al., 2010; Zhang et al., 2006). Sall4 was found to bind regulatory regions of important pluripotency genes such as of *Pou5f1* (previously known as *Oct4*) and *Nanog* (Wu et al., 2006; Zhang et al., 2006) and a physical interaction with the Pou5f1 and Nanog proteins has been reported (Pardo et al., 2010; Rao et al., 2010; van den Berg et al., 2010; Wu et al., 2006). The consensus arising from these studies was that Sall4 is instrumental in the regulation of key pluripotency genes and is thus a key regulator of the pluripotency transcriptional network (van den Berg et al., 2010; Xiong, 2014; Yang et al., 2010). Whether it is essential for self-renewal remains a point of contention.

Sall1 and Sall4 have both been shown to interact biochemically with the Nucleosome Remodelling and Deacetylase (NuRD) complex. NuRD is a transcriptional regulatory complex that has nucleosome remodelling activity due to the Chd4 helicase and protein deacetylase activity due to Hdac1 and Hdac2. Additional NuRD components are the zinc-finger proteins Gatad2a/b, SANT domain proteins Mta1/2/3, histone chaperones Rbbp4/7, structural protein Mbd3 (which can be substituted for by the methyl-CpG-binding protein Mbd2) and the small Cdk2ap1 protein (Allen et al., 2013; Le Guezennec et al., 2006). The usual interpretation of the

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Embryonic Intra-Aortic Clusters Undergo Myeloid Differentiation Mediated by Mesonephros-Derived CSF1 in Mouse

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Abstract The aorta-gonad-mesonephros (AGM) region contains intra-aortic clusters (IACs) thought to have acquired hematopoietic stem cell (HSC) potential in vertebrate embryos. To assess extrinsic regulation of IACs in the AGM region, we employed mouse embryos harboring a *Sall1-GFP* reporter gene, which allows identification of mesonephros cells based on GFP expression. Analysis of AGM region tissue sections confirmed mesonephros GFP expression. Mesonephric cells sorted at E10.5 expressed mRNA encoding *Csf1*, a hematopoietic cytokine, and corresponding protein, based on real-time PCR and immunocytochemistry, respectively. Further analysis indicated that some IACs express the CSF1 receptor, CSF1R. Expression of *Cebpa* and *Irf8* mRNAs was higher in CSF1R-positive IACs, whereas that of *Cebpε* and *Gfi1* mRNAs was lower relative to CSF1R-negative IACs, suggesting that CSF1/CSF1R signaling functions in IAC myeloid differentiation by modulating expression of these transcription factors. Colony formation assays using CSF1R-positive IACs revealed increased numbers of myeloid colonies in the presence of CSF1. Analysis using an intra-cellular signaling array indicated the greatest fold increase of Cleaved Caspase-3 in AGM cells in the presence of CSF1. Immunohistochemistry revealed that Cleaved Caspase-3 is primarily expressed in IACs in the AGM region, and incubation of IACs with CSF1 up-regulated Cleaved Caspase-3. Overall, our findings suggest that CSF1 secreted from mesonephros accelerates IAC myeloid differentiation in the AGM region, possibly via Caspase-3 cleavage.

Keywords Intra-aortic clusters · Hematopoietic stem cells · AGM region · Mesonephros · CSF1 · Myeloid differentiation

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Introduction

During mouse embryogenesis, there are two distinct waves of hematopoiesis: primitive hematopoiesis, which gives rise to transient progenitor populations that differentiate into primitive erythrocytes and macrophages [1–6], and definitive hematopoiesis, which sustains the blood system through hematopoietic stem cells (HSCs) capable of reconstituting adult bone marrow hematopoiesis [6, 7]. Cells capable of reconstituting neonatal recipients, known as "pre-HSCs", are detected in the para-aortic-splanchnopleural mesoderm (P-Sp)/Aorta-Gonad-Mesonephros (AGM) region and to a lesser

Cell Reports
Article

Selective In Vitro Propagation of Nephron Progenitors Derived from Embryos and Pluripotent Stem Cells

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SUMMARY

Nephron progenitors in the embryonic kidney propagate while generating differentiated nephrons. However, in mice, the progenitors terminally differentiate shortly after birth. Here, we report a method for selectively expanding nephron progenitors in vitro in an undifferentiated state. Combinatorial and concentration-dependent stimulation with LIF, FGF2/9, BMP7, and a WNT agonist is critical for expansion. The purified progenitors proliferated beyond the physiological limits observed in vivo, both for cell numbers and lifespan. Neonatal progenitors were maintained for a week, while progenitors from embryonic day 11.5 expanded 1,800-fold for nearly 20 days and still reconstituted 3D nephrons containing glomeruli and renal tubules. Furthermore, progenitors generated from mouse embryonic stem cells and human induced pluripotent cells could be expanded with retained nephron-forming potential. Thus, we have established in vitro conditions for promoting the propagation of nephron progenitors, which will be essential for dissecting the mechanisms of kidney organogenesis and for regenerative medicine.

INTRODUCTION

A mammalian kidney contains a large number of nephrons (approximately one million in humans), which are functional units consisting of glomeruli and renal tubules. The kidney is formed by reciprocally inductive interactions, starting at embryonic day 10.5 (E10.5) to E11.5 in mice, between two precursor lineages the metanephric mesenchyme (MM) and the ureteric bud. The former contains nephron progenitors that express the transcription factor SIX2 (Kobayashi et al., 2008; Self et al., 2006), and these cells give rise to nephron epithelia in response to the canonical wingless-type mouse mammary tumor virus integration site family member (WNT) signal evoked by ureteric bud-derived *Wnt9b* (Carroll et al., 2005) and subsequent non-canonical signals including the $Ca2^{+}$-dependent pathway (Tanigawa et al., 2011). *Six2* opposes the *Wnt*-mediated differentiation signal, thereby maintaining nephron progenitors in the undifferentiated state (Park et al., 2012). Thus, the balance between propagation and differentiation of nephron progenitors is important for kidney organogenesis. Many other transcription factors, including *Sall1* (Kanda et al., 2014; Osafune et al., 2006), *Pax2* (Ranghini and Dressler, 2015), *Wt1* (Kann et al., 2015; Motamedi et al., 2014), and *Osr1* (Xu et al., 2014), are also involved in the maintenance of nephron progenitors, and their cooperative network is being elucidated. Nephron progenitors begin to express CITED1, which marks the most undifferentiated population, at E13.5, a few days after their initial appearance. Therefore, the CITED1+/SIX2+ fraction represents naive nephron progenitors from E13.5.

Nephron progenitors cease propagation and are terminally differentiated within a few days after birth in mice (Hartman et al., 2007; Short et al., 2014) and at 34 weeks of gestation in humans (Tank et al., 2012). In mice, a burst of differentiation occurs after birth, which leads to the formation of multiple nephrons per ureteric bud tip with altered distal-proximal patterning (Rumballe et al., 2011). Thus, no nephron formation occurs in the adult kidney, which may underlie the irreversible nature of diseased kidneys. Considering that nephron progenitors are formed at E10.5–11.5 in mice, they are maintained only for 10 days in vivo. Recently, Chen et al. reported that even the CITED1+ population changed its character during development and heterogeneity increased in older (postnatal day 0 [P0]) populations (Chen et al., 2015). Their data suggest that there are no age-resistant stem cells and that the majority of the cap mesenchymal cells intrinsically age in vivo. However, older cells were rejuvenated when transplanted in a younger niche, and cell-cell contacts are required for rejuvenation. Therefore, the niche environment may, at least partly, override the intrinsic aging of nephron progenitors. Thus, releasing them from their limited expansion will contribute greatly to the understanding of kidney development. It will also be beneficial for emerging regenerative medicine, because several groups, including ours, established the protocols to generate nephron progenitors from mouse embryonic stem cells (ESCs) and/or human induced pluripotent stem (iPSCs) (Taguchi et al., 2014; Takasato et al., 2014, 2015; Morizane et al., 2015).

The pluripotency factor *Nanog* regulates pericentromeric heterochromatin organization in mouse embryonic stem cells

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An open and decondensed chromatin organization is a defining property of pluripotency. Several epigenetic regulators have been implicated in maintaining an open chromatin organization, but how these processes are connected to the pluripotency network is unknown. Here, we identified a new role for the transcription factor NANOG as a key regulator connecting the pluripotency network with constitutive heterochromatin organization in mouse embryonic stem cells. Deletion of *Nanog* leads to chromatin compaction and the remodeling of heterochromatin domains. Forced expression of NANOG in epiblast stem cells is sufficient to decompact chromatin. NANOG associates with satellite repeats within heterochromatin domains, contributing to an architecture characterized by highly dispersed chromatin fibers, low levels of H3K9me3, and high major satellite transcription, and the strong transactivation domain of NANOG is required for this organization. The heterochromatin-associated protein SALL1 is a direct cofactor for NANOG, and loss of *Sall1* recapitulates the *Nanog*-null phenotype, but the loss of *Sall1* can be circumvented through direct recruitment of the NANOG transactivation domain to major satellites. These results establish a direct connection between the pluripotency network and chromatin organization and emphasize that maintaining an open heterochromatin architecture is a highly regulated process in embryonic stem cells.

[*Keywords*: embryonic stem cells; pluripotency; heterochromatin; nuclear organization]

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The genome of eukaryotic cells is organized into euchromatin, which is generally permissive for gene transcription and activation, and heterochromatin, which is largely gene-poor. This form of nuclear compartmentalization is thought to impact genome regulation and stability, thereby contributing to cell identity (Fraser and Bickmore 2007; Misteli 2007; Bickmore and van Steensel 2013). Pluripotent mouse embryonic stem cell (ESC) chromatin exists in an unusual configuration with widely dispersed open chromatin throughout the nucleoplasm, including within constitutive heterochromatin domains such as pericentromeric satellite repeats (Meshorer et al. 2006; Efroni et al. 2008; Fussner et al. 2011; de Wit et al. 2013). A similar form of highly dispersed chromatin architecture also characterizes pluripotent epiblast cells within

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Short communication

Sall1 transiently marks undifferentiated heart precursors and regulates their fate

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ABSTRACT

Cardiac progenitor cells (CPCs) are a crucial source of cells in cardiac development and regeneration. However, reported CPCs are heterogeneous, and no gene has been identified to transiently mark undifferentiated CPCs throughout heart development. Here we show that *Spalt-like gene 1* (*Sall1*), a zing-finger transcription factor, is expressed in undifferentiated CPCs giving rise to both left and right ventricles. Sall1 was transiently expressed in precardiac mesoderm contributing to the first heart field (left ventricle precursors) but not in the field itself. Similarly, Sall1 expression was maintained in the second heart field (outflow tract/right ventricle precursors) but not in cardiac cells. In vitro, high levels of Sall1 at mesodermal stages enhanced cardiomyogenesis, whereas its continued expression suppressed cardiac differentiation. Our study demonstrates that Sall1 marks CPCs in an undifferentiated state and regulates cardiac differentiation. These findings provide fundamental insights into CPC maintenance, which can be instrumental for CPC-based regenerative medicine.

1. Introduction

Cardiac progenitor cells (CPCs), identified from embryos or pluripotent stem cell (PSC) culture, hold tremendous regenerative potential with the unique capability to expand and differentiate into cardiac cells [1]. Cardiogenesis initiate as the basic-helix-loop-helix factor Mesp1 is expressed in precardiac mesoderm. Mesp1^{+} cells migrate and form the cardiac crescent (referred to as the first heart field (FHF)), which largely contribute to the left ventricle (LV). A subset of Mesp1^{+} cells remain undifferentiated in the second heart field (SHF), located dorsal to the FHF, and give rise to the outflow tract (OFT), right ventricle (RV), and part of atria [2]. Early CPCs can be identified by transient expression of the chromatin factor Smarcd3, prior to expression of known CPC markers [3, 4], including Islet1 (Isl1), fetal liver kinase 1 (Flk1), and Nkx2.5 [1]. While Isl1 is transiently expressed in the SHF, Smarcd3 and Nkx2-5 are continually expressed in cardiomyocytes [5, 6]. Although, CPCs can be identified by their stage-specific expression of various cell markers, no gene has been identified to mark undifferentiated pools of CPCs giving rise to four chambers throughout development. In the present study, we demonstrate that Sall1, a zinc-finger transcription factor, is transiently expressed and maintained in undifferentiated CPCs throughout early heart development in vivo. Sall1 has a biphasic role: early overexpression of Sall1 enhanced, but its late overexpression suppressed cardiomyogenesis in pluripotent stem cell (PSC) culture.

2. Materials and methods

2.1. Mice and lineage tracing

Sall1^{GFP}, *Sall1^{CreERT2}*, *ROSA^{YFP}* mouse lines were generated as described [7, 8]. Experiments were conducted according to a protocol approved by the International Animal Care and Use Committee of IMCB, the University

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REVIEW

Induction of nephron progenitors and glomeruli from human pluripotent stem cells

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Abstract Studies of kidney regeneration using stem cells have progressed rapidly in recent years. Our group has developed a protocol to induce nephron progenitors from both mouse and human pluripotent stem cells which is based on a revised model of early stage kidney specification. The induced progenitors readily reconstitute three-dimensional nephron structures, including glomeruli and renal tubules, in vitro. We can further generate human induced pluripotent stem cells (iPSCs), in which nephrin-expressing glomerular podocytes are tagged with green fluorescent protein (GFP). The sorted GFP-positive cells retain the podocyte-specific molecular and structural features. Upon transplantation, mouse endothelial cells of the host animals are integrated into the human iPSC-derived glomeruli, and the podocytes show further maturation. Other laboratories have reported different protocols to induce nephron structures from human iPSCs in vitro. These findings will accelerate our understanding of kidney development and diseases in humans.

Keywords Nephron progenitor · Six2 · Sall1 · Glomerulus · Podocyte · Nephrin

Introduction

Studies in the field of stem cell biology for kidney reconstruction have progressed rapidly in recent years. This review is based on our presentation at the International Workshop for Developmental Nephrology in 2015 and, therefore, we mainly focus on the progress we have made in our own research, although we also include studies from other research groups. We discuss future perspectives based on all current findings, but the reader should refer to the original publications of the different research groups included in this review for more details on these specific studies.

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Starting from the frog kidney

The nephron is the basic functional unit of the kidney and includes a glomerulus and a renal tubule. The adult kidney contains approximately 1 million nephrons, and their three-dimensional (3D) structures are required for the kidney to function. Additionally, the kidney takes three forms during development, i.e., the pronephros, mesonephros, and metanephros, which further complicates kidney generation strategies in vitro. The pronephros and the majority of the mesonephros eventually degenerate, and the metanephros becomes the permanent kidney in mammals. The animal cap, which is a small portion of blastula stage *Xenopus* embryos, can be differentiated into pronephric tubules within 3 days by treatment with activin + retinoic acid. We used this animal cap system to identify genes expressed in the pronephros and potentially in the mesonephros and metanephros [1]. One of the identified genes was homologous to the *Drosophila* region-specific homeotic gene *spalt* (*sal*), which contains multiple double zinc-finger motifs [2]. We then isolated a mouse homolog (*Sall1*) and found that *Sall1* knockout mice show kidney agenesis [3]. Humans and mice each have four known *sal*-like genes, and mutations in *SALL1* and *SALL4* are associated with autosomal dominant diseases that involve abnormalities in various appendages and organs, including the ears, limbs, heart, and

STEM CELLS AND RENAL DEVELOPMENT IN 2015

Advances in generating and maintaining nephron progenitors

Ryuichi Nishinakamura

2015 saw the publication of several important studies in the renal stem cell and developmental biology fields. Key studies provided insights into the ageing of nephron progenitors and optimal conditions to stimulate the expansion of nephron progenitors, and reported the *in vitro* generation of kidney organoids.

The kidney develops through mutual interactions between two precursor tissues — the metanephric mesenchyme and the ureteric bud. The metanephric mesenchyme contains nephron progenitors that give rise to the epithelia of glomeruli and renal tubules whereas the ureteric bud gives rise to the collecting ducts and ureters. Nephron progenitors, which typically express the markers *Six2* and *Cited1*, proliferate and are maintained in an immature state at the tips of the branching ureteric bud; some nephron progenitors, however, differentiate into nephron epithelia in response to signals from the ureteric buds (Fig. 1). This balanced process between progenitor propagation and differentiation continues during development, and is a critical determinant of nephron number, which might affect kidney function in adult life. All remaining nephron progenitors undergo differentiation shortly after birth in mice and before birth in humans. As nephron progenitors are established at embryonic day 10 in mice, they are maintained only for ~10 days *in vivo*.

> “intrinsic age-dependent changes affect cell–cell interactions”

A new paper[1] shows that nephron progenitors age during development. Single-cell sequencing revealed that gene expression profiles of mouse nephron progenitors differ depending on their developmental stage, and that older progenitor populations (postnatal day 0 in this case) contain a more heterogeneous population of cells than do younger populations (embryonic day 12.5). Age-dependent increases in mTor activity and ribosome biogenesis and a reduction in Fgf20 levels were also observed. When the researchers transplanted nephron progenitors into the progenitor region (niche) located at the periphery of the developing kidney, they found that the older progenitors tended to exit from the niche and differentiate more quickly than the younger progenitors. Interestingly, some of the older progenitors remained in the niche (that is, they behaved more like the younger progenitors), when transplanted into a niche comprising young progenitors. This alteration in behaviour of older progenitors might in part be explained by an abundant secretion of Fgf20 by the young progenitors. These results suggest that intrinsic age-dependent changes affect cell–cell interactions, which may cause the cessation of nephrogenesis around the time of birth. In other words, nephron progenitors *in vivo* are not ‘self-renewing’ like age-resistant stem cells, but rather should be considered to be transient amplifying cells.

These findings lead to the question of whether nephron progenitors can be induced to ‘self-renew’ artificially — an issue that was tackled, and partially resolved, by Brown *et al.*[2]. These researchers found that inhibition of Bmp-dependent Smad activity through use of a small molecule inhibitor could maintain murine nephron progenitors. Activation of several other pathways, including Wnt and Fgf-regulated pathways, led to further expansion of the nephron progenitors for >20 days *in vitro*, even from neonatal progenitors that readily differentiate *in vivo*. The *in vitro*-expanded cells retained expression of progenitor-related markers, such as *Cited1* and *Six2*, and retained the potential to differentiate into renal tubule epithelia with features of proximo–distal nephron

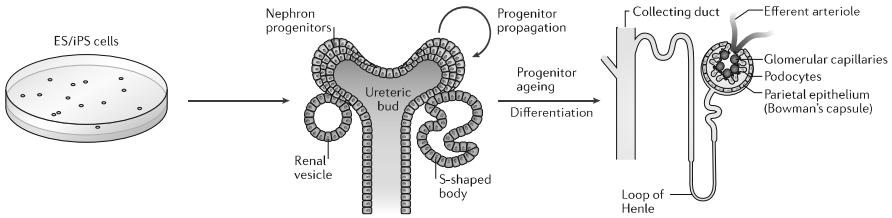

Figure 1 | **Strategies towards the generation, propagation and differentiation of nephron progenitors.** Nephron progenitors have been shown to age *in vivo*, and interact with the ureteric bud to form the nephron. Nephron progenitors can now be induced from pluripotent stem cells, and propagated *in vitro* with at least partial differentiation potential. ES, embryonic stem; iPS, induced pluripotent stem.

BASIC RESEARCH www.jasn.org

Human Induced Pluripotent Stem Cell–Derived Podocytes Mature into Vascularized Glomeruli upon Experimental Transplantation

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ABSTRACT

Glomerular podocytes express proteins, such as nephrin, that constitute the slit diaphragm, thereby contributing to the filtration process in the kidney. Glomerular development has been analyzed mainly in mice, whereas analysis of human kidney development has been minimal because of limited access to embryonic kidneys. We previously reported the induction of three-dimensional primordial glomeruli from human induced pluripotent stem (iPS) cells. Here, using transcription activator–like effector nuclease-mediated homologous recombination, we generated human iPS cell lines that express green fluorescent protein (GFP) in the *NPHS1* locus, which encodes nephrin, and we show that GFP expression facilitated accurate visualization of nephrin-positive podocyte formation *in vitro*. These induced human podocytes exhibited apicobasal polarity, with nephrin proteins accumulated close to the basal domain, and possessed primary processes that were connected with slit diaphragm–like structures. Microarray analysis of sorted iPS cell–derived podocytes identified well conserved marker gene expression previously shown in mouse and human podocytes *in vivo*. Furthermore, we developed a novel transplantation method using spacers that release the tension of host kidney capsules, thereby allowing the effective formation of glomeruli from human iPS cell–derived nephron progenitors. The human glomeruli were vascularized with the host mouse endothelial cells, and iPS cell–derived podocytes with numerous cell processes accumulated around the fenestrated endothelial cells. Therefore, the podocytes generated from iPS cells retain the podocyte-specific molecular and structural features, which will be useful for dissecting human glomerular development and diseases.

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The glomerulus is the filtering apparatus of the kidney and contains three types of cells: podocytes, vascular endothelial cells, and mesangial cells. Podocytes cover the basal domains of the endothelial cells *via* the basement membrane and play a major role in the filtration process.[1,2] Podocytes possess multiple cytoplasmic protrusions. The primary processes are complicated by the further stemming of smaller protrusions (secondary processes or foot processes), which interdigitate with those from neighboring podocytes. The gaps between these foot processes are connected with the slit diaphragm, which is detectable only by electron microscopy. The molecular nature of the slit diaphragm was initially revealed by identification of *NPHS1* as the gene responsible for Finnish-type

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S.S. and A.T. contributed equally to this work.

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Sall1 in renal stromal progenitors non-cell autonomously restricts the excessive expansion of nephron progenitors

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The mammalian kidney develops from reciprocal interactions between the metanephric mesenchyme and ureteric bud, the former of which contains nephron progenitors. The third lineage, the stroma, fills up the interstitial space and is derived from distinct progenitors that express the transcription factor Foxd1. We showed previously that deletion of the nuclear factor *Sall1* in nephron progenitors leads to their depletion in mice. However, *Sall1* is expressed not only in nephron progenitors but also in stromal progenitors. Here we report that specific *Sall1* deletion in stromal progenitors leads to aberrant expansion of nephron progenitors, which is in sharp contrast with a nephron progenitor-specific deletion. The mutant mice also exhibited cystic kidneys after birth and died before adulthood. We found that *Decorin*, which inhibits Bmp-mediated nephron differentiation, was upregulated in the mutant stroma. In contrast, the expression of *Fat4*, which restricts nephron progenitor expansion, was reduced mildly. Furthermore, the Sall1 protein binds to many stroma-related gene loci, including *Decorin* and *Fat4*. Thus, the expression of *Sall1* in stromal progenitors restricts the excessive expansion of nephron progenitors in a non-cell autonomous manner, and *Sall1*-mediated regulation of *Decorin* and *Fat4* might at least partially underlie the pathogenesis.

A typical mammalian kidney contains approximately one million nephrons, which are functional units consisting of glomeruli, proximal and distal renal tubules, and collecting ducts. During development, the nephron is formed by reciprocally inductive interactions between two precursor tissues: the metanephric mesenchyme and the ureteric bud. The former contains nephron progenitors that express the transcription factor Six2, and give rise to most components of the nephron epithelia, including those in glomeruli (podocytes and parietal cells) and renal tubules[1]. In contrast, the collecting duct epithelium is derived from the ureteric bud. However, these two lineages are not sufficient to generate the complete kidney structure. A third lineage—the stroma—is required, which is derived from a distinct progenitor population that expresses the transcription factor Foxd1[2,3]. The stromal progenitors surround the Six2-positive nephron progenitors, and are maintained in the outermost cortical region of the kidney during development. The interstitial tissue between the nephron's epithelia is filled with differentiated stromal cells, such as fibroblasts, pericytes, and glomerular mesangial cells, the latter two being closely associated with vasculature.

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Preferential Propagation of Competent SIX2+ Nephronic Progenitors by LIF/ROCKi Treatment of the Metanephric Mesenchyme

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SUMMARY

Understanding the mechanisms responsible for nephrogenic stem cell preservation and commitment is fundamental to harnessing the potential of the metanephric mesenchyme (MM) for nephron regeneration. Accordingly, we established a culture model that preferentially expands the MM SIX2+ progenitor pool using leukemia inhibitory factor (LIF), a Rho kinase inhibitor (ROCKi), and extracellular matrix. Passaged MM cells express the key stem cell regulators *Six2* and *Pax2* and remain competent to respond to WNT4 induction and form mature tubular epithelia and glomeruli. Mechanistically, LIF activates STAT, which binds to a *Stat* consensus sequence in the *Six2* proximal promoter and sustains SIX2 levels. ROCKi, on the other hand, attenuates the LIF-induced differentiation activity of JNK. Concomitantly, the combination of LIF/ROCKi upregulates *Slug* expression and activates YAP, which maintains SIX2, PAX2, and SALL1. Using this novel model, our study underscores the pivotal roles of SIX2 and YAP in MM stem cell stability.

INTRODUCTION

Although considerable progress has been made in understanding the cues that direct self-renewal and differentiation of pluripotent stem cells (Buehr et al., 2008), the factors and pathways capable of perpetuating any multipotent tissue-specific progenitor in the absence of immortalizing genetic modifications remain largely undefined. During development, reciprocal interactions between the ureteric bud (UB) and the surrounding metanephric mesenchyme (MM) direct the formation of the metanephros. The MM promotes the branching morphogenesis of the UB to generate the collecting duct network. In turn, the UB induces condensation and mesenchymal-epithelial transition (MET) in the MM to initiate nephron formation at each bud tip. Condensed cells of the MM cap the tips of the branching UB in the cortical nephrogenic zone of the metanephros and provide a self-renewing population of SIX2+ progenitors, which supply the precursors for nephronic epithelia (Kobayashi et al., 2008). Ablation of *Six2* results in the premature commitment of these progenitors and a depletion of the progenitor pool. Therefore, SIX2 is a major determinant in the maintenance and self-renewal of the nephronic precursor. The aggregate SIX2-expressing population is further regulated by the transcriptional co-activator and Hippo pathway component Yes-associated protein (YAP) and is growth-limited by signals emanating from the encapsulating cortical stroma (Das et al., 2013). The loss of stromal signals promotes the expansion of undifferentiated SIX2+ stem cells, stimulates the nuclear localization of YAP, and inhibits the formation of nephronic structures. Conversely, *Yap* ablation causes renal hypoplasia, characterized by a measureable deficit in progenitor self-renewal and fewer nephrons. These findings led us to hypothesize that constitutive activation of SIX2 and YAP is sufficient to sustain this tissue-specific stem cell.

During development, extrinsic signals in a progenitor's microenvironment provide cues for self-renewal and lineage commitment. Although several growth factors, including fibroblast growth factors (FGFs) 2 (Perantoni et al., 1995), 8 (Perantoni et al., 2005), 9, and 20 (Barak et al., 2012) and epidermal growth factor (EGF)/transforming growth factor α (TGF-α) (Rogers et al., 1992), support the survival of MM cells and facilitate the limited expansion of this population in culture, they have proven to be insufficient for long-term propagation of progenitors with stem-like properties and nephronic potential. In this study, we optimize the niche for rat progenitors using growth factors, extracellular matrix, and Rho kinase inhibitor, which, in combination, sustain SIX2 and YAP nuclear expression. Moreover, we demonstrate that these factors contribute to the preferential propagation and partial stabilization of MM progenitors with the preservation of stem cell markers and a capacity for differentiation.

RESULTS

The Extracellular Matrix Helps Stabilize MM Progenitors

Primary cultures of MM were generated from developmentally comparable embryonic day (E) 13.5 rat or E11.5 mouse

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Notch1 and Notch2 in Podocytes Play Differential Roles During Diabetic Nephropathy Development

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Notch pathway activation in podocytes has been shown to play an important role in diabetic kidney disease (DKD) development; however, the receptors and ligands involved in the process have not been identified. Here, we report that conditional deletion of Notch1 in podocytes using NPHS2^{cre}Notch1$^{flox/flox}$ animals resulted in marked amelioration of DKD. On the contrary, podocyte-specific genetic deletion of Notch2 had no effect on albuminuria and mesangial expansion. Notch1-null podocytes were protected from apoptosis and dedifferentiation in vitro, likely explaining the protective phenotype in vivo. Deletion of Notch1 in podocytes also resulted in an increase in Notch2 expression, indicating an interaction between the receptors. At the same time, transgenic overexpression of Notch2 in podocytes did not induce phenotypic changes, while constitutive expression of Notch1 caused rapid development of albuminuria and glomerulosclerosis. In summary, our studies indicate that Notch1 plays a distinct (nonredundant) role in podocytes during DKD development.

To date, approximately 9% of the U.S. population has diabetes. Diabetes is the leading cause of chronic kidney disease and end-stage renal failure. Diabetic kidney disease (DKD) is initiated in part because high level of blood glucose damages the glomerular filtration unit, resulting in protein leakage into the urine (1). The filtration unit is comprised of capillary endothelial cells, glomerular basement membrane, and specialized epithelial cells, podocytes. Once thought to be primarily quiescent and terminally differentiated cells, podocytes have been demonstrated to be the true culprit of DKD (1). Podocyte injury is characterized by pathological loss of regularity in foot branching and widening of the foot processes; changes termed "foot process effacement." Foot process effacement is the typical mechanism of injury response of podocytes, and it is usually associated with a broader dedifferentiation process. Severe insult leads to podocyte loss by apoptosis or detachment (1). Reactivation of developmental pathways, including Wnt and Notch signaling, has been shown to play an important role in podocyte injury and DKD development by promoting dedifferentiation and apoptosis (2,3).

The Notch protein family is comprised of four receptors, Notch1–4, and five canonical ligands, Jagged1 and -2 (Jag1 and -2) and delta-like ligands (Dll)1, -3, and -4 (4). Canonical Notch signaling is typically transcellular; the ligand(s) expressed on one cell binds to receptors on neighboring cells and initiates cleavage of the receptor. Notch cleavage results in the release of the Notch intracellular domain (NICD or ICNotch), which translocates to the nucleus to become a transcriptional coregulator. Some of the transcriptional binding partners that engage NICD in the nucleus are common to all Notch receptors; including mastermind-like 1 (MAML1) and recombination signal binding protein for immunoglobulin kappa J (Rbpjκ) (4). Despite the common use of activation and signaling partners, Notch receptor functions are often nonredundant (5).

Notch1 and Notch2 show high structural similarities and an almost overlapping expression pattern in the developing and adult mammalian kidney. Despite their intersecting expression, Notch1 and -2 are functionally distinct. Mutations of NOTCH2 in patients cause Alagille

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Sall2 is required for proapoptotic Noxa expression and genotoxic stress-induced apoptosis by doxorubicin

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The Sall2 transcription factor is deregulated in several cancers; however, little is known about its cellular functions, including its target genes. Recently, we demonstrated that p53 directly regulates Sall2 expression under genotoxic stress. Here, we investigated the role of Sall2 in the context of cellular response to genotoxic stress. In addition, we further examined the Sall2-p53 relationship during genotoxic stress in primary mouse embryo fibroblasts (MEFs), which are derived from *Sall2* knockout mice separately, or in combination with the *p53ERTAM* knock-in mice. We found that the levels of Sall2 mRNA and protein are dynamically modulated in response to doxorubicin. At early times of stress, Sall2 is downregulated, but increases under extension of the stress in a p53-independent manner. Based on caspase-3/7 activities, expression of cleaved poly (ADP-ribose) polymerase, expression of cleaved caspase-3 and induction of proapoptotic proteins, Sall2 expression was correlated with cellular apoptosis. Consequently, *Sall2*$^{-/-}$ MEFs have decreased apoptosis, which relates with increased cell viability in response to doxorubicin. Importantly, Sall2 was required for apoptosis even in the presence of fully activated p53. Searching for putative Sall2 targets that could mediate its role in apoptosis, we identified proapoptotic *NOXA/PMAIP1* (phorbol-12-myristate-13-acetate-induced protein 1). We demonstrated that Sall2 positively regulates *Noxa* promoter activity. Conserved putative Sall2-binding sites at the *NOXA* promoter were validated *in vitro* by electrophoretic mobility shift assay and *in vivo* by ChIP experiments, identifying *NOXA* as a novel Sall2 target. In agreement, induction of Noxa protein and mRNA in response to doxorubicin was significantly decreased in *Sall2*$^{-/-}$ MEFs. In addition, studies in leukemia Jurkat T cells support the existence of the Sall2/Noxa axis, and the significance of this axis on the apoptotic response to doxorubicin in cancer cells. Our study highlights the relevance of Sall2 in the apoptotic response to extended genotoxic stress, which is important for understanding its role in normal physiology and disease.
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Deregulation of the Sall2 transcription factor is associated with the development of human diseases, including cancer, ocular coloboma and Alport syndrome.[1–4] However, Sall2 normal function, regulation and immediate target genes are not well known, making it difficult to understand its role in various diseases.

SALL2 is a member of the Spalt/Sal family of transcription factors characterized by their role in organ development and conserved from *Caenorhabditis elegans* to humans.[5–7] *Sall2*-deficient mice were previously reported to have no apparent abnormal phenotype when bred on a C57BL/6 genetic background; however, a strain-specific incidence of neural tube defects and perinatal lethality were reported when bred on mixed genetic backgrounds.[8,9] Recently, it was demonstrated that Sall2 has a role in eye morphogenesis and a *SALL2* gene mutation was associated with coloboma, a congenital eye defect.[2,10] A deleterious *SALL2* mutation was also associated with Alport syndrome, a renal disease,[1] suggesting that Sall2 could have a role in kidney development.

Evidences for Sall2 association with cancer are increasing, but are still controversial. Several studies suggest a tumor suppressor role for Sall2 in ovarian cancer[3,11,12] and in primary acute myeloid leukemia.[13] However, Sall2 is found upregulated in Wilm's tumor,[14] synovial sarcoma,[15,16] oral cancer[17,18] and testicular cancer,[19] and is one of the four neurodevelopmental transcription factors essential for glioblastoma propagation.[4] The molecular mechanisms underlying the role of Sall2 as a tumor suppressor in certain types of cancer and its deregulation in others are still unknown.

To understand the role of Sall2 in normal and disease states, it is essential to define Sall2 targets under different cell contexts. Sall2 targets identified to date include the cell cycle regulatory gene *p21*^{WAF} (protein 21 wild-type p53 activation

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Abbreviations: 4-OHT, 4-hydroxytamoxifen; BAX, BCL2-associated X protein; BAD, BCL2-associated death promoter; BCL2, B-cell lymphoma 2; BH3, BCL2 homology domain 3; ChIP, chromatin immunoprecipitation; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, dimethyl sulfoxide; EMSA, electrophoretic mobility shift assay; ER/ER, estrogen receptor; EtOH, ethanol; FBS, fetal bovine serum; GFP, green fluorescent protein; HOSE, human ovarian surface epithelial; MEF, mouse embryo fibroblast; MG132, carbobenzoxy-Leu-Leu-leucinal; p21^{WAF}, protein 21 wild-type p53 activation factor; PARP, poly (ADP-ribose) polymerase; *PMAIP1*, phorbol-12-myristate-13-acetate-induced protein 1
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A mouse model of Townes-Brocks syndrome expressing a truncated mutant Sall1 protein is protected from acute kidney injury

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Hirsch S, El-Achkar T, Robbins L, Basta J, Heitmeier M, Nishinakamura R, Rauchman M. A mouse model of Townes-Brocks syndrome expressing a truncated mutant Sall1 protein is protected from acute kidney injury. *Am J Physiol Renal Physiol* 309: F852–F863, 2015. First published August 26, 2015; doi:10.1152/ajprenal.00222.2015.—It has been postulated that developmental pathways are reutilized during repair and regeneration after injury, but functional analysis of many genes required for kidney formation has not been performed in the adult organ. Mutations in *SALL1* cause Townes-Brocks syndrome (TBS) and nonsyndromic congenital anomalies of the kidney and urinary tract, both of which lead to childhood kidney failure. Sall1 is a transcriptional regulator that is expressed in renal progenitor cells and developing nephrons in the embryo. However, its role in the adult kidney has not been investigated. Using a mouse model of TBS ($Sall1^{TBS}$), we investigated the role of *Sall1* in response to acute kidney injury. Our studies revealed that Sall1 is expressed in terminally differentiated renal epithelia, including the S3 segment of the proximal tubule, in the mature kidney. $Sall1^{TBS}$ mice exhibited significant protection from ischemia-reperfusion injury and aristolochic acid-induced nephrotoxicity. This protection from acute injury is seen despite the presence of slowly progressive chronic kidney disease in $Sall1^{TBS}$ mice. Mice containing null alleles of *Sall1* are not protected from acute kidney injury, indicating that expression of a truncated mutant protein from the $Sall1^{TBS}$ allele, while causative of congenital anomalies, protects the adult kidney from injury. Our studies further revealed that basal levels of the preconditioning factor heme oxygenase-1 are elevated in $Sall1^{TBS}$ kidneys, suggesting a mechanism for the relative resistance to injury in this model. Together, these studies establish a functional role for Sall1 in the response of the adult kidney to acute injury.

acute kidney injury; Sall1; Townes-Brocks syndrome; renal hypoplasia; nephrotoxicity

EVERY YEAR, ~17 MILLION HOSPITALIZED Americans are treated for acute kidney injury (AKI) (6), with an associated mortality rate as high as 52–80% (3, 7). Patients that survive AKI have significant associated morbidity and an increase in 30-day mortality after hospital discharge (4, 6, 36, 50). In the longer term, any incidence of clinically detectable AKI increases the risk for progressive chronic kidney disease (CKD) (6). Despite the high incidence and complications of AKI, treatment options remain largely supportive due to a lack of understanding of the mechanisms governing repair and recovery from AKI.

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Sall1 is a transcriptional regulator that is essential for kidney development (33). Truncating (nonsense) mutations in *SALL1* cause Townes-Brock syndrome (TBS; OMIM 107480), an autosomal dominant congenital disorder that is associated with defects in multiple organs, including the kidney (21). Missense mutations in *SALL1* contribute to a relatively high percentage of nonsyndromic congenital anomalies of the kidney and urinary tract, a common cause of childhood kidney failure (16, 48). These findings highlight the importance of *SALL1* in human kidney development and disease.

Studies in mouse mutants have demonstrated that *Sall1* is required to control the balance between self-renewal and differentiation of nephron progenitors during formation of the kidney (2, 18). It has been postulated that developmental pathways are reutilized in regeneration of adult tissues. However, the role of tissue-restricted transcription factors such as Sall1 in normal cell turnover and in repair/regeneration after injury of differentiated renal epithelia is not well understood. In this study, we investigated the role of Sall1 in the adult kidney in a basal state and in response to acute injury. Our data reveal that Sall1 is expressed in renal tubular epithelia and plays an important role in the protective response to ischemic and toxic insults to the kidney.

METHODS

Mouse strains. $Sall1^{TBS}$, $Sall1^{GFP}$, $Sall1^{flox}$, and β-*actin*-Cre:ER mouse strains have been previously described (14, 19, 42, 53). The genomic structure of these three *Sall1* mutant alleles and their protein products are shown in Fig. 1. $Sall1^{TBS}$ and $Sall1^{GFP}$ mice used in this study are heterozygous mutants. Homozygous mutants of both these strains die in the early postnatal period due to kidney failure as a result of severe renal hypoplasia or agenesis. $Sall1^{TBS}$ mice produce a truncated Sall1 N-terminal protein which mimics that found in TBS; $Sall1^{GFP}$ is a null allele that produces green fluorescent protein (GFP) under the control of the *Sall1* genomic locus. $Sall1^{cKO}$ mice were generated by crossing homozygous $Sall1^{flox/flox}$ mice to β-*actin*-Cre:ER, $Sall1^{flox/+}$ mice, yielding $Sall1^{flox/flox}$ β-*actin*-Cre:ER +/− mice. Lactating mothers were administered 5 mg/kg tamoxifen in corn oil by intraperitoneal injection at postnatal *day 1* and *2* to activate Cre. Cre-mediated deletion resulted in homozygous postnatal deletion of Sall1 exon 1, thereby creating a null allele; we refer to this allele as $Sall1^{cKO}$ mice. Deletion of *Sall1* was confirmed by quantitative (q) PCR and immunostaining.

AKI. All animal studies were approved by the Veterans Affairs St. Louis Health Care System Institutional Animal Care and Use Committee (IACUC) and conducted in conformity with the *Guiding Principles for Research Involving Animals and Human Beings*. Renal ischemia-reperfusion injury (IRI) was performed as previously described (11). Eight-week-old male *Sall1* mutant mice and wild-type

Sall4-Gli3 system in early limb progenitors is essential for the development of limb skeletal elements

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Limb skeletal elements originate from the limb progenitor cells, which undergo expansion and patterning to develop each skeletal element. Posterior-distal skeletal elements, such as the ulna/fibula and posterior digits develop in a Sonic hedgehog (*Shh*)-dependent manner. However, it is poorly understood how anterior-proximal elements, such as the humerus/femur, the radius/tibia and the anterior digits, are developed. Here we show that the zinc finger factors *Sall4* and *Gli3* cooperate for proper development of the anterior-proximal skeletal elements and also function upstream of *Shh*-dependent posterior skeletal element development. Conditional inactivation of *Sall4* in the mesoderm before limb outgrowth caused severe defects in the anterior-proximal skeletal elements in the hindlimb. We found that *Gli3* expression is reduced in *Sall4* mutant hindlimbs, but not in forelimbs. This reduction caused posteriorization of nascent hindlimb buds, which is correlated with a loss of anterior digits. In proximal development, *Sall4* integrates *Gli3* and the *Plzf-Hox* system, in addition to proliferative expansion of cells in the mesenchymal core of nascent hindlimb buds. Whereas forelimbs developed normally in *Sall4* mutants, further genetic analysis identified that the *Sall4-Gli3* system is a common regulator of the early limb progenitor cells in both forelimbs and hindlimbs. The *Sall4-Gli3* system also functions upstream of the *Shh*-expressing ZPA and the *Fgf8*-expressing AER in fore- and hindlimbs. Therefore, our study identified a critical role of the *Sall4-Gli3* system at the early steps of limb development for proper development of the appendicular skeletal elements.

Sall4 | Gli3 | limb progenitors | appendicular skeletal elements | Plzf-Hox

How progenitor cells are spatially and temporarily organized to construct an organ is a central question in developmental biology. Limb skeletal elements develop from limb progenitors, which arise from the lateral plate mesoderm (LPM) that is originated from epithelial somatopleure (1). Limb progenitors initially form two paired protrusions, fore- and hindlimb buds, whose initiation occurs around embryonic day (E) 9.0 and E9.5, respectively, in mouse embryos. In the following steps, limb signaling centers, known as the zone of polarizing activity (ZPA) and apical ectodermal ridge (AER), are established. SHH (Sonic hedgehog) from the ZPA and FGF8 from the AER are major signal molecules that regulate proliferative expansion and patterning of early limb progenitor cells (reviewed in ref. 2). These processes lead to development of functional limbs with each skeletal element adopting a unique shape at a distinct location.

Several studies suggest that limb progenitors consist of two distinct pools, an anterior progenitor pool and a posterior progenitor pool. The posterior progenitor pool consists of cells that once expressed *Shh* and cells that received paracrine effects of SHH, which contribute to digit 2 (d2), d3, d4, and d5 and the posterior zeugopod (ulna, fibula) (3–6). Contrary to this, the anterior progenitors are not well characterized. However, a recent report suggested that the putative anterior progenitor pool contributes to d1 and anterior-proximal skeletal elements (tibia, femur) in hindlimbs. Inactivating *Irx3* and *Irx5* (*Irx3/5*), two homeodomain encoding genes expressed in the LPM and the anterior-proximal domain of developing fore- and hindlimb buds, resulted in failure to develop these anterior-proximal skeletons in hindlimbs, whereas forelimbs developed normally (7). The lack of the anterior-proximal hindlimb skeleton was observed only when *Irx3/5* were inactivated before hindlimb initiation, suggesting that the putative anterior-progenitor pool is specified before hindlimb outgrowth.

In addition, limb anterior genes appear to have a critical role in establishing limb signaling centers. One of factors involved in this process is a zinc finger factor GLI3 (8). *Gli3* functions for specifying anterior-posterior polarity in the nascent limb buds (9), and *Gli3* null embryos develop polydactyly without polarity (10). Furthermore, simultaneous inactivation of *Gli3* and *Irx3/5* caused failure to express *Shh* and significantly reduced levels of *Fgf8*, which resulted in severe limb truncation (11). This result suggests that cooperation of anterior genes is upstream of establishing limb signaling centers, and therefore expansion of SHH-dependent posterior progenitors. This phenotype was observed only in the hindlimbs, although *Irx3/5* and *Gli3* are similarly expressed in both fore- and hindlimb buds. Despite these recent reports, factors that regulate the putative anterior progenitors are not well understood. Moreover, it is unknown whether similar or distinct mechanisms involving anterior genes regulate limb signaling center establishment in fore- and hindlimbs.

Sall genes encode zinc finger transcription factors that are vertebrate homologs of the *Drosophila* homeotic gene, *spalt*. They play diverse roles in embryonic development, including the development of limbs (12, 13). Among four *Sall* genes in mammals, *Sall4* is a key regulator of stemness in stem cells and progenitor cells, such as embryonic stem cells, induced pluripotent stem cells, spermatogenial progenitor cells and cancer cells (14–19). Despite its robust expression during limb development, functions of *Sall4*

Significance

The limb skeletal elements that have unique morphology and distinct locations are developed from limb progenitors, derived from the lateral plate mesoderm. These skeletal elements arise during limb development. In this study, we show genetic evidence that function of *Sall4* is essential prior to limb outgrowth for development of the anterior-proximal skeletal elements. Furthermore, genetic interaction between *Sall4* and *Gli3* is upstream of establishing *Shh* (Sonic hedgehog) expression, and therefore, *Shh*-dependent posterior skeletal elements. Our study identified early requirements of the *Sall4-Gli3* system for two putative progenitor pools that develop into distinct sets of limb skeletal elements.

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http://www.kidney-international.org

Nephron reconstitution from pluripotent stem cells

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It has been a challenge in developmental biology and regenerative medicine to generate nephron progenitors that reconstitute the three-dimensional (3D) nephron structure *in vitro*. Many studies have tried to induce nephron progenitors from pluripotent stem cells by mimicking the developmental processes *in vivo*. However, the current developmental model does not precisely address the spatiotemporal origin of nephron progenitors, hampering our understanding of cell fate decisions in the kidney. Here, we present a revised model of early-stage kidney specification, suggesting distinct origins of the two major kidney components: the ureteric bud and metanephric mesenchyme. This model enables the induction of metanephric nephron progenitors from both mouse and human pluripotent stem cells. The induced cells self-organize in the presence of Wnt signaling and reconstitute 3D nephron structures including both nephric tubules with a clear lumina and glomeruli with podocytes. The engrafted kidney tissue develops vascularized glomeruli and nephric tubules, but it does not produce urine, suggesting the requirement for further maturation. Nevertheless, the generation of nephron components from human-induced pluripotent stem cells will be useful for future application in regenerative therapy and modeling of congenital kidney diseases *in vitro*. This review discusses the possibility of *de novo* organogenesis of a functional kidney from pluripotent stem cells and the future direction toward clinical applications.

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KEYWORDS: development; intermediate mesoderm; kidney; origin; regenerative medicine; stem cell

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STRATEGIES FOR REGENERATIVE MEDICINE OF THE KIDNEY

Increasing number of patients are suffering from chronic kidney disease caused by diabetes, hypertension, glomerulonephritis, polycystic kidney diseases, and other disorders. However, no curative treatments have been established other than renal transplantation. Moreover, chronic kidney disease not only has a risk of mortality but also causes an economic burden. However, recent progress in developmental biology and stem cell biology is realizing the potential of regenerative medicine.[1]

The phrase 'regenerative medicine' implies several perspectives such as tissue repair and *de novo* organogenesis. In terms of tissue repair, one strategy involves the use of the endogenous adult stem cell population of the organ. If such cells can be expanded and then differentiated into the desired cell types, they would be a useful resource for cell therapy. However, the mammalian adult kidney is one of the typical organs that does not possess a robust regenerative capacity. In the research fields of *Drosophila* and zebrafish, the existence of stem cells has been shown in the adult kidneys.[2,3] On the other hand, it is unclear whether the adult kidney in mammals contains such multipotent stem cells.[4] Recently, intrinsic renal tubular epithelial cells, which can regenerate renal epithelium after acute kidney injury, have been identified by lineage trace experiments.[5] Other studies have revealed that epithelial cells within Bowman's capsule possess the capacity to reverse the loss of podocytes,[6] and the dedifferentiation and proliferative capacity of adult podocytes.[7] However, these adult cells possess limited potentials compared with the embryonic nephron progenitors, which are multipotent and give rise to all the epithelial nephron components.[8,9] Another strategy for tissue repair is the *in situ* direct reprograming of cells into the desired cell types by introducing key transcription factors. For other organs, including the pancreas and heart, the core factors are well defined for efficient conversion to desired cell types.[10,11] However, a similar strategy applied to the kidney has not generated fully competent renal cells.[12]

For *de novo* organogenesis, the availability of pluripotent stem cells is essential. Because there has been establishment and propagation of pluripotent stem cells, such as embryonic stem cells[13] and induced pluripotent stem cells (iPSCs),[14] the reconstitution of organs *in vitro* has become more realistic. Many attempts to induce defined cell types from such pluripotent stem cells have been made during the past few decades. The most common approach is recapitulation of the key signals required for lineage specification during

Sexually dimorphic expression of *Mafb* regulates masculinization of the embryonic urethral formation

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Masculinization of external genitalia is an essential process in the formation of the male reproductive system. Prominent characteristics of this masculinization are the organ size and the sexual differentiation of the urethra. Although androgen is a pivotal inducer of the masculinization, the regulatory mechanism under the control of androgen is still unknown. Here, we address this longstanding question about how androgen induces masculinization of the embryonic external genitalia through the identification of the v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog B (Mafb) gene. *Mafb* is expressed prominently in the mesenchyme of male genital tubercle (GT), the anlage of external genitalia. MAFB expression is rarely detected in the mesenchyme of female GTs. However, exposure to exogenous androgen induces its mesenchymal expression in female GTs. Furthermore, MAFB expression is prominently down-regulated in male GTs of *androgen receptor* (*Ar*) KO mice, indicating that AR signaling is necessary for its expression. It is revealed that *Mafb* KO male GTs exhibit defective embryonic urethral formation, giving insight into the common human congenital anomaly hypospadias. However, the size of *Mafb* KO male GTs is similar with that of wild-type males. Moreover, androgen treatment fails to induce urethral masculinization of the GTs in *Mafb* KO mice. The current results provide evidence that *Mafb* is an androgen-inducible, sexually dimorphic regulator of embryonic urethral masculinization.

Mafb | masculinization | urethra | hypospadias | androgen receptor

External genitalia exhibit marked sexual dimorphism in mammals. The organ size of external genitalia is longer in male mice than female mice. Androgen-dependent control of organ size is widely accepted as a masculinization parameter. In addition to androgen-dependent size control, mesenchymal differentiation is an essential process of masculinization to form sexually dimorphic structures such as corporal tissues, the penile bone, and the urethra. Sexual differentiation of corporal tissues and penile bones occurs after birth (1, 2). In contrast, urethral formation shows sexual dimorphism during embryogenesis (Fig. 1) (3). Androgen actions are central to masculinization of external genitalia (4, 5). However, the mechanisms mediating androgen-dependent masculinization processes are largely unknown. Failure of exposure to androgen causes lack of masculinization of male genital tubercles (GTs) (3, 6). Therefore, proper timing of embryonic exposure to androgen is essential for inducing GT masculinization. Androgen actions are mediated through a specific receptor, the androgen receptor (AR). Recent genetic studies on a conditional allele of *Ar* suggest that mesenchymal androgen actions are essential for GT masculinization (3).

Embryonic masculinization processes of male GTs are divided into androgen-dependent organ size control and androgen-dependent mesenchymal differentiation to form the urethra. Researchers have focused on the identification of masculinization genes under the control of androgen actions for many years. However, such genes have not been identified. Recently, growth factor signals have been suggested to regulate the masculinization of the size of GTs (3, 7). However, the modulation of such growth factor signals does not induce the urethral masculinization (3, 7). These observations indicate that distinct regulators may induce embryonic urethral masculinization under androgen actions. Thus, it is still unclear how such unique morphogenetic processes are mediated by androgen-driven regulators.

The external genitalia are the most common sites of congenital abnormalities in humans (8–10). Of those, hypospadias is the frequent abnormality in which the urethral opening is ectopically located on the ventral (lower) side of the penis (8, 10–12). The male urethra connects the bladder to the penis and transports urine and semen outside of the body. In males, the urethra is incorporated into the glans to form the tubular urethra (Fig. 1). The urethral plate is an early, transitory developmental structure that develops into the tubular urethra in males. Fusion of the urethral plate is an essential process for tubular urethral formation (13). Various environmental substances are speculated to modulate developmentally essential hormonal pathways. Maternal exposure to estrogenic compounds may increase the risk of hypospadias because these compounds may interfere with the effects of fetal androgen (14–16). These studies indicate that hypospadias may be caused by disruption of the androgen exposure required for embryonic urethral masculinization. The

DEVELOPMENTAL BIOLOGY

Significance

Androgen is essential for the masculinization of external genitalia such as the organ size and the male-type urethra in mammals. However, the genes downstream of androgen, which are responsible for these masculinization processes, have not been identified. Here, we show v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog B (*Mafb*) as an essential masculinization gene for embryonic urethral formation. *Mafb* expression is prominent in developing male external genitalia, driving masculinization of embryonic urethral formation in an androgen-dependent manner. External genitalia of *Mafb* KO males exhibit urethral defects, giving insight into human hypospadias. The current findings indicate that *Mafb* is a crucial mediator of urethral masculinization and is a possible new candidate gene for hypospadias derived from embryonic abnormalities.

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STEM CELLS

TISSUE-SPECIFIC STEM CELLS

Sall4 Is Essential for Mouse Primordial Germ Cell Specification by Suppressing Somatic Cell Program Genes

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Key Words. Primordial germ cells • Spalt-like 4 • HDAC • Ifitm1 • Mouse

ABSTRACT

The Spalt-like 4 (Sall4) zinc finger protein is a critical transcription factor for pluripotency in embryonic stem cells (ESCs). It is also involved in the formation of a variety of organs, in mice, and humans. We report the essential roles of Sall4 in mouse primordial germ cell (PGC) specification. PGC specification is accompanied by the activation of the stem cell program and repression of the somatic cell program in progenitor cells. Conditional inactivation of Sall4 during PGC specification led to a reduction in the number of PGCs in embryonic gonads. *Sall4*$^{del/del}$ PGCs failed to translocate from the mesoderm to the endoderm and underwent apoptosis. In *Sall4*$^{del/del}$ PGC progenitors, somatic cell program genes (*Hoxa1* and *Hoxb1*) were derepressed, while activation of the stem cell program was not impaired. We demonstrated that in differentiated ESCs, Sall4 bound to these somatic cell program gene loci, which are reportedly occupied by Prdm1 in embryonic carcinoma cells. Given that Sall4 and Prdm1 are known to associate with the histone deacetylase repressor complex, our findings suggest that Sall4 suppresses the somatic cell program possibly by recruiting the repressor complex in conjunction with Prdm1; therefore, it is essential for PGC specification. STEM CELLS *2015;33:289–300*

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INTRODUCTION

Progenitor cell formation of mouse primordial germ cells (PGCs) begins with activation of *Prdm1* (also known as *Blimp1*) in a subset of epiblast cells in the proximal region of the pre-gastrulation embryo. At the streak stage, progenitor cells form a cellular cluster and express *Prdm1* along with *interferon-induced transmembrane protein* (*Ifitm*)*−3* (also known as *mil-1/fragilis*) at the base of allantois in the posterior mesoderm of the embryo. PGCs are specified in the progenitor cell cluster at embryonic day (E) 7.25. This is accompanied by their translocation from the mesoderm to the endoderm for subsequent migration toward the genital ridges [1–5]. Specification of PGCs is directed by transcriptional network regulation. Activation of "germ/stem cell program" genes, such as *Pou5f1* (also known as *oct-3/4*), *Nanog*, *Sox2*, and *Dppa3* (also known as *Pgc7/stella*), and the repression of "somatic cell program" genes, such as *Hoxa1* and *Hoxb1*, determines which progenitor cells become PGCs [2, 3, 6, 7]. Prdm1 is known to have a dominant function in repression of the somatic program during PGC specification, but its precise mechanisms remain poorly understood.

The stemness-associated factor, *spalt-like 4* (*Sall4*), a mammalian homolog of the *Drosophila* homeotic gene *spalt*, encodes a zinc finger nuclear protein. *SALL4* is also known as the gene responsible for Okihiro syndrome, characterized by a range of congenital disorders that affect the eyes, ears, heart, kidney, limbs, and other organs [8, 9]. In mice, inactivation of *Sall4* leads to embryonic lethality immediately after implantation, and Sall4 haploinsufficiency partially phenocopies Okihiro syndrome [10–12].

In embryonic stem cells (ESCs) and extra-embryonic endoderm (XEN) cells, Sall4 contributes to the regulation of stemness as a transcriptional repressor and activator [13–15]. It is likely that the function of Sall4 might be defined by its partner binding protein(s). As a transcriptional repressor, Sall4 forms a protein complex with a silencer complex, such as the class I histone deacetylase (HDAC) complex, and contributes to the repression of "differentiation promoting" genes [15, 16]. As a transcriptional activator, Sall4 forms a protein complex with pluripotency factors such as Pou5f1, Nanog, and Sox2, activating genes involved in stem cell maintenance [13, 14, 17–19]. Sall4 also regulates the stem cell

Nonmuscle Myosin II Regulates the Morphogenesis of Metanephric Mesenchyme–Derived Immature Nephrons

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ABSTRACT

The kidney develops from reciprocal interactions between the metanephric mesenchyme and ureteric bud. The mesenchyme transforms into epithelia and forms complicated nephron structures, whereas the ureteric bud extends its pre-existing epithelial ducts. Although the roles are well established for extracellular stimuli, such as Wnt and Notch, it is unclear how the intracellular cytoskeleton regulates these morphogenetic processes. *Myh9* and *Myh10* encode nonmuscle myosin II heavy chains, and *Myh9* mutations in humans are implicated in congenital kidney diseases and focal segmental glomerulosclerosis in adults. Here, we analyzed the roles of *Myh9* and *Myh10* in the developing kidney. Ureteric bud-specific depletion of *Myh9* resulted in no apparent phenotypes, whereas mesenchyme-specific *Myh9* deletion caused proximal tubule dilations and renal failure. Mesenchyme-specific *Myh9*/*Myh10* mutant mice died shortly after birth and showed a severe defect in nephron formation. The nascent mutant nephrons failed to form a continuous lumen, which likely resulted from impaired apical constriction of the elongating tubules. In addition, nephron progenitors lacking *Myh9*/*Myh10* or the possible interactor *Kif26b* were less condensed at midgestation and reduced at birth. Taken together, nonmuscle myosin II regulates the morphogenesis of immature nephrons derived from the metanephric mesenchyme and the maintenance of nephron progenitors. Our data also suggest that *Myh9* deletion in mice results in failure to maintain renal tubules but not in glomerulosclerosis.

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The kidney develops from reciprocal induction between two precursor tissues: the mesenchyme and ureteric bud. At embryonic day 11.5 (E11.5) in the mouse, the ureteric bud invades the metanephric mesenchyme, and Wnt9b secreted from the bud induces Six2-positive nephron progenitors in the mesenchyme to transform into epithelia.[1,2] The differentiating cells lose Six2 expression and begin to express Wnt4, which further enhances differentiation.[3] After the immature epithelia emerge, Notch2 signaling specifies the nascent nephrons into the distal and proximal regions.[4,5] Thus, progenitors sequentially transit to pretubular aggregates, renal vesicles, and then, C- and S-shaped bodies, which eventually develop into nephron

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Dullard/Ctdnep1 Regulates Endochondral Ossification via Suppression of TGF-β Signaling

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ABSTRACT

Transforming growth factor (TGF)-β signaling plays critical roles during skeletal development and its excessive signaling causes genetic diseases of connective tissues including Marfan syndrome and acromelic dysplasia. However, the mechanisms underlying prevention of excessive TGF-β signaling in skeletogenesis remain unclear. We previously reported that *Dullard/Ctdnep1* encoding a small phosphatase is required for nephron maintenance after birth through suppression of bone morphogenetic protein (BMP) signaling. Unexpectedly, we found that *Dullard* is involved in suppression of TGF-β signaling during endochondral ossification. Conditional *Dullard*-deficient mice in the limb and sternum mesenchyme by *Prx1-Cre* displayed the impaired growth and ossification of skeletal elements leading to postnatal lethality. *Dullard* was expressed in early cartilage condensations and later in growth plate chondrocytes. The tibia growth plate of newborn *Dullard* mutant mice showed reduction of the proliferative and hypertrophic chondrocyte layers. The sternum showed deformity of cartilage primordia and delayed hypertrophy. Micromass culture experiments revealed that *Dullard* deficiency enhanced early cartilage condensation and differentiation, but suppressed mineralized hypertrophic chondrocyte differentiation, which was reversed by treatment with TGF-β type I receptor kinase blocker LY-364947. *Dullard* deficiency induced upregulation of protein levels of both phospho-Smad2/3 and total Smad2/3 in micromass cultures without increase of *Smad2/3* mRNA levels, suggesting that Dullard may affect Smad2/3 protein stability. The phospho-Smad2/3 level was also upregulated in perichondrium and hypertrophic chondrocytes in *Dullard*-deficient embryos. Response to TGF-β signaling was enhanced in *Dullard*-deficient primary chondrocyte cultures at late, but not early, time point. Moreover, perinatal administration of LY-364947 ameliorated the sternum deformity in vivo. Thus, we identified Dullard as a new negative regulator of TGF-β signaling in endochondral ossification. © 2014 American Society for Bone and Mineral Research.

KEY WORDS: DULLARD/CTDNEP1; PRX1-CRE; TGF-β; CHONDROCYTES; ENDOCHONDRAL OSSIFICATION

Introduction

Endochondral ossification is a key developmental process by which bones grow. In a first step, mesenchyme condensation occurs, which is followed by chondrocyte differentiation. The chondrocytes then proliferate, undergo hypertrophy, and terminally differentiate into mineralized cartilage, which is subsequently replaced by bone. Several lines of evidence demonstrate that multiple signaling pathways are involved in these well-coordinated steps.[1]

Transforming growth factor (TGF)-β signaling plays diverse roles in cellular differentiation, proliferation, and cancer. In early skeletal development, mouse genetic studies have shown that TGF-β signaling is required for sternum formation, perichondrium development, and joint morphogenesis.[2–6] On the other hand, excessive TGF-β signaling is associated with congenital connective tissue diseases, including Marfan syndrome,[7] Loeys-Dietz syndrome,[8–10] and Shprintzen-Goldberg syndrome,[11] which are characterized by craniofacial and skeletal deformity as well as aortic aneurysm. On the other hand, the acromelic dysplasia group, including Weill-Marchesani syndrome,[12,13] geleophysic dysplasia,[14] acromicric dysplasia,[15] and Myhre syndrome,[16] also involves increased TGF-β signaling.[17] These diseases are characterized by short statue and short bones, in marked contrast to bone overgrowth as seen in Marfan and its related disorders. These studies suggest that limiting TGF-β signaling is also crucial to coordinate connective tissue development and homeostasis. However, the precise mechanism of suppression of TGF-β signaling in normal skeletal development is not well understood.

Dullard (also called *Ctdnep1*) encodes a small C-terminal domain phosphatase with putative transmembrane domain at

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Sall1 Maintains Nephron Progenitors and Nascent Nephrons by Acting as Both an Activator and a Repressor

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ABSTRACT

The balanced self-renewal and differentiation of nephron progenitors are critical for kidney development and controlled, in part, by the transcription factor Six2, which antagonizes canonical Wnt signaling-mediated differentiation. A nuclear factor, Sall1, is expressed in Six2-positive progenitors as well as differentiating nascent nephrons, and it is essential for kidney formation. However, the molecular functions and targets of Sall1, especially the functions and targets in the nephron progenitors, remain unknown. Here, we report that *Sall1* deletion in Six2-positive nephron progenitors results in severe progenitor depletion and apoptosis of the differentiating nephrons in mice. Analysis of mice with an inducible *Sall1* deletion revealed that Sall1 activates genes expressed in progenitors while repressing genes expressed in differentiating nephrons. Sall1 and Six2 co-occupied many progenitor-related gene loci, and Sall1 bound to Six2 biochemically. In contrast, Sall1 did not bind to the *Wnt4* locus suppressed by Six2. Sall1-mediated repression was also independent of its binding to DNA. Thus, Sall1 maintains nephron progenitors and their derivatives by a unique mechanism, which partly overlaps but is distinct from that of Six2: Sall1 activates progenitor-related genes in Six2-positive nephron progenitors and represses gene expression in Six2-negative differentiating nascent nephrons.

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The nephron is a basic functional unit of the kidney, which includes the glomerulus, proximal and distal renal tubules, and the loop of Henle. The mammalian kidney, the metanephros, is formed by reciprocally inductive interactions between two precursor tissues, namely the metanephric mesenchyme and the ureteric bud. The mesenchyme contains nephron progenitors that express a transcription factor, Six2. When Six2-positive cells are labeled using *Six2GFPCre*, a mouse strain expressing Cre recombinase fused to green fluorescent protein (GFP) under the control of the *Six2* promoter, they give rise to nephron epithelia *in vivo*.[1] *Six2* opposes the canonical Wnt-mediated differentiation evoked by ureteric bud-derived *Wnt9b*, thereby maintaining the self-renewal of nephron progenitors.[2–4] However, the progenitors gradually lose *Six2* expression

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Cellular and Molecular Life Sciences

REVIEW

Regulation of male sex determination: genital ridge formation and *Sry* activation in mice

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Abstract Sex determination is essential for the sexual reproduction to generate the next generation by the formation of functional male or female gametes. In mammals, primary sex determination is commenced by the presence or absence of the Y chromosome, which controls the fate of the gonadal primordium. The somatic precursor of gonads, the genital ridge is formed at the mid-gestation stage and gives rise to one of two organs, a testis or an ovary. The fate of the genital ridge, which is governed by the differentiation of somatic cells into Sertoli cells in the testes or granulosa cells in the ovaries, further determines the sex of an individual and their germ cells. Mutation studies in human patients with disorders of sex development and mouse models have revealed factors that are involved in mammalian sex determination. In most of mammals, a single genetic trigger, the Y-linked gene *Sry* (sex determination region on Y chromosome), regulates testicular differentiation. Despite identification of *Sry* in 1990, precise mechanisms underlying the sex determination of bipotential genital ridges are still largely unknown. Here, we review the recent progress that has provided new insights into the mechanisms underlying genital ridge formation as well as the regulation of *Sry* expression and its functions in male sex determination of mice.

Keywords Six1 · Six4 · Sox9 · Transcriptional network · Nr5a1/Ad4BP/Sf1

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Abbreviations

Alpl/Akp2/TNAP	Alkaline phosphatase, liver/bone/kidney
AR	Androgen receptor
Arx	X-linked *aristaless*-related homeobox
ATR-X	Alpha thalassemia, mental retardation, X-linked
Cbln4	Cerebellin precursor 4
Cbx2/M33	Chromobox homolog 2/mouse polycomb group member M33
ChIP	Chromatin immunoprecipitation
Col9a3	Collagen, type IX, alpha 3
Cyp17a1	Cytochrome P450, family 17, subfamily a, polypeptide 1
Cyp26b1	Cytochrome P450, family 26, subfamily b, polypeptide 1
Dax1/Nr0b1	Dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1/nuclear receptor subfamily 0 group B, member 1
dazl	Deleted in azoospermia-like
Dhh/Ptch1	Desert hedgehog/patched 1
Dmrt1	*Doublesex* and *mab-3* related transcription factor 1
DSD	Disorders of sex development
E	Embryonic day
EG cells	Embryonic germ cells
EGF	Epidermal growth factor
Eif2s3y	Eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked
EMT	Epithelial mesenchymal transition
Emx2	*Empty spiracles* homeobox 2

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Role of Wnt5a-Ror2 Signaling in Morphogenesis of the Metanephric Mesenchyme during Ureteric Budding

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Development of the metanephric kidney begins with the induction of a single ureteric bud (UB) on the caudal Wolffian duct (WD) in response to GDNF (glial cell line-derived neurotrophic factor) produced by the adjacent metanephric mesenchyme (MM). Mutual interaction between the UB and MM maintains expression of GDNF in the MM, thereby supporting further outgrowth and branching morphogenesis of the UB, while the MM also grows and aggregates around the branched tips of the UB. Ror2, a member of the Ror family of receptor tyrosine kinases, has been shown to act as a receptor for Wnt5a to mediate noncanonical Wnt signaling. We show that Ror2 is predominantly expressed in the MM during UB induction and that *Ror2*- and *Wnt5a*-deficient mice exhibit duplicated ureters and kidneys due to ectopic UB induction. During initial UB formation, these mutant embryos show dysregulated positioning of the MM, resulting in spatiotemporally aberrant interaction between the MM and WD, which provides the WD with inappropriate GDNF signaling. Furthermore, the numbers of proliferating cells in the mutant MM are markedly reduced compared to the wild-type MM. These results indicate an important role of Wnt5a-Ror2 signaling in morphogenesis of the MM to ensure proper epithelial tubular formation of the UB required for kidney development.

Ror2 is a member of the Ror family of receptor tyrosine kinases and acts as a receptor or coreceptor for Wnt5a to activate a noncanonical Wnt signaling pathway and to inhibit the canonical Wnt signaling pathway (1–4). Wnt5a-Ror2 signaling primarily regulates cell polarity and migration in a variety of cell types (1, 5–11). Wnt5a-Ror2 signaling also plays a crucial role in maintaining neural progenitor cells in a proliferative, undifferentiated state in the developing neocortex (12). During mouse development, *Ror2* and *Wnt5a* are expressed in various tissues and organs in spatiotemporally similar manners (13–15). Furthermore, *Ror2*- and *Wnt5a*-deficient mice exhibit overall similarities in their phenotypes (1, 15–18). In humans, mutations within the *Ror2* gene cause an autosomal-recessive form of Robinow syndrome (RRS), which is characterized by short stature, mesomelic limb shortening, brachydactyly, vertebral abnormalities, and a typical fetal face (19, 20). Importantly, several renal abnormalities, including double ureters and kidneys, hydronephrosis, and rudimentary kidney, have been reported to be associated with RRS (21, 22). However, the role of Wnt5a-Ror2 signaling in kidney development is largely unknown.

Development of the metanephric kidney depends on proper interactions between the ureteric epithelium and metanephric mesenchyme (MM) (23). In mice, it begins at embryonic day 10.5 (E10.5) to E11.0, when a single ureteric bud (UB) emerges from the caudal Wolffian duct (WD) and invades the dorsally localized MM. The UB then grows and undergoes branching morphogenesis to form the collecting duct system, while the MM aggregates around the branched tips of the UB and undergoes mesenchymal-epithelial transition and tubulogenesis to form nephrons. Defects in these early induction events can result in congenital anomalies of the kidney and urinary tract (CAKUT), a major cause of renal failure in children (24). Although mutations within several genes have been identified as the cause of human CAKUT, little is known about the molecular pathogenesis of these disorders (25). Accumulating evidence demonstrates that GDNF (glial cell line-derived neurotrophic factor), a growth factor that is produced by the MM, plays central roles in UB induction, acting via its receptor, Ret, expressed in the WD (26–31). Several mechanisms have been reported to regulate GDNF-Ret signaling during UB formation: the transcription factor FoxC1 and Slit2/Robo2 signaling repress the rostral expression of *Gdnf* (32, 33), while Sprouty1, an intracellular inhibitor of receptor tyrosine kinase signaling, reduces the sensitivity of the WD to GDNF (34). Aberrant regulation of GDNF-Ret signaling might result in ectopic activation of GDNF-Ret signaling, leading to ectopic formation of the UBs in the rostral WD.

Here, we show that Ror2 is mainly expressed in the MM during the initiation of UB formation and that both *Ror2*- and *Wnt5a*-deficient embryos exhibit ectopic UBs, resulting in the formation of duplicated ureters and kidneys. Furthermore, loss of *Ror2* or *Wnt5a* causes spatiotemporally aberrant interaction between the MM and WD due to abnormal positioning of the MM, thereby providing the WD with inappropriate GDNF signaling. These re-

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Nonmyocytic Androgen Receptor Regulates the Sexually Dimorphic Development of the Embryonic Bulbocavernosus Muscle

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The bulbocavernosus (BC) is a sexually dimorphic muscle observed only in males. Androgen receptor knockout mouse studies show the loss of BC formation. This suggests that androgen signaling plays a vital role in its development. Androgen has been known to induce muscle hypertrophy through satellite cell activation and myonuclei accretion during muscle regeneration and growth. Whether the same mechanism is present during embryonic development is not yet elucidated. To identify the mechanism of sexual dimorphism during BC development, the timing of morphological differences was first established. It was revealed that the BC was morphologically different between male and female mice at embryonic day (E) 16.5. Differences in the myogenic process were detected at E15.5. The male BC possesses a higher number of proliferating undifferentiated myoblasts. To identify the role of androgen signaling in this process, muscle-specific androgen receptor (AR) mutation was introduced, which resulted in no observable phenotypes. Hence, the expression of AR in the BC was examined and found that the AR did not colocalize with any muscle markers such as Myogenic differentiation 1, Myogenin, and paired box transcription factor 7. It was revealed that the mesenchyme surrounding the BC expressed AR and the BC started to express AR at E15.5. AR mutation on the nonmyocytic cells using spalt-like transcription factor 1 (Sall1) Cre driver mouse was performed, which resulted in defective BC formation. It was revealed that the number of proliferating undifferentiated myoblasts was reduced in the Sall1 Cre:AR$^{L-/Y}$ mutant embryos ,and the adult mutants were devoid of BC. The transition of myoblasts from proliferation to differentiation is mediated by cyclin-dependent kinase inhibitors. An increased expression of p21 was observed in the BC myoblast of the Sall1 Cre:AR$^{L-/Y}$ mutant and wild-type female. Altogether this study suggests that the nonmyocytic AR may paracrinely regulate the proliferation of myoblast possibly through inhibiting p21 expression in myoblasts of the BC. **(*Endocrinology* 155: 2467–2479, 2014)**

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Abbreviations: AGD, anogenital distance; AR, androgen receptor; ARKO, AR knockout; BC, bulbocavernosus; Cdk, cyclin-dependent kinase; CKI, Cdk inhibitor; 3D, three-dimensional; E, embryonic day; EdU, 5-ethynyl-2′-deoxyuridine; GT, genital tubercle; LA, levator ani; MCK, muscle creatine kinase; MPC, muscle precursor cell; MyoD, myogenic differentiation 1; Myog, myogenin; Pax7, paired box transcription factor 7; PDGFRa, platelet-derived growth factor receptor-α; PFA, paraformaldehyde; PIC, PW1+Pax7− interstitial cell; Sall1, spalt-like transcription factor 1; Tfm, testicular-feminization mutation; TM, tamoxifen; P, postnatal day; WT, wild type; YFP, yellow fluorescent protein.

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Preformed Wolffian duct regulates Müllerian duct elongation independently of canonical Wnt signaling or Lhx1 expression

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ABSTRACT The Müllerian duct gives rise to female reproductive organs, such as the oviduct and uterus. During gestation, the Wolffian duct, which generates male reproductive organs and the kidney, is formed, and the Müllerian duct then elongates caudally along the preformed Wolffian duct. Anatomical separation of these two ducts in chick embryos demonstrated that the Wolffian duct is required for Müllerian duct formation. Likewise, a few reports supported this notion in mice, including studies on *Wnt9b* mutant mice and Wolffian duct-specific *Lhx1* deletion. However, anatomical ablation of the Wolffian duct has not been established in mice. In this study, we addressed the importance of the interaction between these two reproductive ducts, by generating mice that specifically expressed a diphtheria toxin subunit in the Wolffian duct. While this genetic ablation of the Wolffian duct resulted in kidney hypoplasia/agenesis in both male and female mutant mice, the female mutant mice lacked the uterus, which is derived from the Müllerian duct. At mid-gestation, the Müllerian duct was truncated at the level where the mutant Wolffian duct was prematurely terminated, meaning that Müllerian duct elongation was dependent on the preformed Wolffian duct. However, *Wnt9b* expression in the Wolffian duct and the resultant canonical Wnt activity, as well as Lhx1 expression, were not affected in the mutant mice. These results suggest that the Wolffian duct regulates Müllerian duct elongation by currently unidentified mechanisms that are independent of canonical Wnt signaling or Lhx1 expression.

KEY WORDS: *Wölffian duct, Müllerian duct, Wnt9b, Lhx1*

It has been shown that a subset of human patients with uterus hypoplasia display kidney hypoplasia (Oppelt *et al.*, 2007; Woolf and Allen, 1953). While the uterus and the kidney might partially employ similar developmental programs, an interaction between the precursor tissues may also be involved in the formation of these two organs. The reproductive organs in males and females are derived from the Wolffian duct and Müllerian duct, respectively (Kobayashi and Behringer, 2003). The Wolffian duct (mesonephric duct) is first detected at embryonic day (E) 8.5 in mice, and elongates caudally until it reaches the cloaca by E10.5. At E10.5, the ureteric bud stems out from the Wolffian duct, and contributes to the formation of the kidney through a mutual interaction with the metanephric mesenchyme (Nishinakamura, 2008). While most parts of the Wolffian duct degenerate during development, the epididymis and vas deferens in males are derived from the residual Wolffian duct. The Müllerian duct (paramesonephric duct) emerges at E11.5 as an invagination of the peritoneal cavity, and elongates along the preformed Wolffian duct. The Müllerian duct elongation is completed when it reaches the urogenital sinus at E13.5. The Müllerian duct eventually forms the oviduct, uterus, and upper third of the vagina in females, while it starts to degenerate in males between E13 and E14 through the effects of Müllerian inhibiting substance, which belongs to the TGF-β superfamily.

The elongation of the Müllerian duct depends on the preformed Wolffian duct. In chicks, physical elimination of the Wolffian duct or separation of the two ducts using aluminum foil or other methods results in impaired elongation of the Müllerian duct (Bishop-Calame, 1966; Kobayashi and Behringer, 2003). In mice, genetic deletion of genes encoding transcription factors, such as *Pax2*, *Lhx1*, and *Emx2*, results in absence of both the Wolffian duct and Müllerian duct (Miyamoto *et al.*, 1997; Shawlot and Behringer, 1995; Torres *et al.*, 1995). Since these genes are expressed in both ducts, it is difficult to discriminate whether the absence of the Müllerian duct is caused by cell-autonomous requirements of these genes in the

Abbreviations used in this paper: E, embryonic day; md, Müllerian duct; wd, Wolffian duct.

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Midline-derived Shh regulates mesonephric tubule formation through the paraxial mesoderm ☆

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ABSTRACT

During organogenesis, Sonic hedgehog (Shh) possesses dual functions: Shh emanating from midline structures regulates the positioning of bilateral structures at early stages, whereas organ-specific Shh locally regulates organ morphogenesis at later stages. The mesonephros is a transient embryonic kidney in amniote, whereas it becomes definitive adult kidney in some anamniotes. Thus, elucidating the regulation of mesonephros formation has important implications for our understanding of kidney development and evolution. In *Shh* knockout (KO) mutant mice, the mesonephros was displaced towards the midline and ectopic mesonephric tubules (MTs) were present in the caudal mesonephros. Mesonephros-specific ablation of *Shh* in *Hoxb7-Cre*;$Shh^{flox/-}$ and $Sall1^{CreERT2/+}$;$Shh^{flox/-}$ mice embryos indicated that *Shh* expressed in the mesonephros was not required for either the development of the mesonephros or the differentiation of the male reproductive tract. Moreover, stage-specific ablation of *Shh* in $Shh^{CreERT2/flox}$ mice showed that notochord- and/or floor plate-derived Shh were essential for the regulation of the number and position of MTs. Lineage analysis of hedgehog (Hh)-responsive cells, and analysis of gene expression in *Shh* KO embryos suggested that Shh regulated nephrogenic gene expression indirectly, possibly through effects on the paraxial mesoderm. These data demonstrate the essential role of midline-derived Shh in local tissue morphogenesis and differentiation.

Introduction

The vertebrate kidney is derived from the intermediate mesoderm (IM), which is a narrow strip of tissue located between the paraxial and lateral plate mesoderm (Schoenwolf, 2000). Depending upon the species, up to three separate kidney structures form in an anterior–posterior sequence during embryonic development (Saxen, 1987). The first to form, and most anterior, is the pronephros, which is the functional embryonic kidney in most fish and amphibians, and a transient embryonic anlage in amniotes. The mesonephros, which is the main embryonic/fetal kidney in amniotes and will become the adult kidney in anamniotes, subsequently forms posterior to the pronephros. The metanephros, which is the last to form and is the most posterior, is specific to amniotes and becomes the definitive adult kidney (Saxen, 1987). Thus, the origin of the adult kidney and the distribution of nephrons along the nephrogenic cord vary across the animal kingdom.

In mice, the mesonephros consists of approximately 11 pairs of mesonephric tubules (MTs) and pretubular mesenchymal condensation extending from the level of somite 10–17, and has distinct cranial and caudal domains (Sainio, 2003; Vetter and Gibley, 1966). Cranial MTs are connected to the Wolffian duct (WD) at 4–6 sites, whereas the caudal MTs, which are the bulk of the mesonephros, are primitive, unbranched tubules that do not connect to the WD. The WD differentiates into the male reproductive tract including the epididymis. MTs in the cranial domain become the efferent duct connecting the epididymis and testis, whereas MTs in the caudal domain regress. MTs first appear as condensations of the nephrogenic cord (Vetter and

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Redefining the In Vivo Origin of Metanephric Nephron Progenitors Enables Generation of Complex Kidney Structures from Pluripotent Stem Cells

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SUMMARY

Recapitulating three-dimensional (3D) structures of complex organs, such as the kidney, from pluripotent stem cells (PSCs) is a major challenge. Here, we define the developmental origins of the metanephric mesenchyme (MM), which generates most kidney components. Unexpectedly, we find that posteriorly located T^+ MM precursors are developmentally distinct from $Osr1^+$ ureteric bud progenitors during the postgastrulation stage, and we identify phasic Wnt stimulation and stage-specific growth factor addition as molecular cues that promote their development into the MM. We then use this information to derive MM from PSCs. These progenitors reconstitute the 3D structures of the kidney in vitro, including glomeruli with podocytes and renal tubules with proximal and distal regions and clear lumina. Furthermore, the glomeruli are efficiently vascularized upon transplantation. Thus, by reevaluating the developmental origins of metanephric progenitors, we have provided key insights into kidney specification in vivo and taken important steps toward kidney organogenesis in vitro.

INTRODUCTION

Despite the successful achievement of various types of tissues from pluripotent stem cells (PSCs), kidney generation in vitro has remained a challenge for developmental biology and regenerative medicine (Murry and Keller, 2008; Williams et al., 2012). This is partly because the specification of the kidney lineage in vivo has not been well clarified, owing to its complex processes involving the formation of three consecutive primordia (pronephros, mesonephros, and metanephros) during embryogenesis (Saxen, 1987). The pronephros and mesonephros eventually degenerate in females, whereas a portion of the mesonephros in males contributes to a drainage system for the future testis, including the epididymis. The kidney derives from the embryonic metanephros, which develops at the most posterior part of the body trunk. The metanephros is formed by reciprocally inductive interactions between two precursor tissues, namely the metanephric mesenchyme (MM) and the ureteric bud. Cell fate analyses have shown that both the MM and ureteric bud derive from the intermediate mesoderm, which appears around embryonic day 8.5 (E8.5) and expresses the transcription factor *Osr1* (Mugford et al., 2008b). However, the mechanisms underlying how the nascent mesoderm becomes committed to the intermediate mesoderm and how the MM and ureteric bud lineage segregate from one another have not been clarified, despite many reports showing the importance of various growth factors during kidney development (Costantini and Kopan, 2010; Fleming et al., 2013; Kim and Dressler, 2005; Moriya et al., 1993; Poladia et al., 2006). Moreover, it remains to be elucidated how the anteroposterior axis is formed along the intermediate mesoderm and gives rise to the posteriorly located metanephros. In the present study, we addressed these questions by in vivo lineage-tracing experiments together with in-vitro-directed differentiation systems utilizing sorted embryonic kidney precursors at each developmental stage. Importantly, by establishing conditions for the later stages first, and then moving backward to the earlier stages, we were able to optimize the multistep culture conditions from embryonic precursors toward nephron progenitors. Finally, the protocol established by these strategies was successfully applied to the induction of metanephric nephron progenitors from mouse embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs), thereby advocating a model for kidney lineage specification.

RESULTS

The $Osr1^+$/$Integrina8^+$/$Pdgfra^-$ Population Represents Colony-Forming Nephron Progenitors

The MM gives rise to the epithelia of glomeruli (i.e., podocytes) and renal tubules, which constitute the major parts of the nephrons, as shown by cell fate analyses involving labeling of

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REVIEW

BMP signaling and its modifiers in kidney development

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Abstract The kidney develops through mutual interactions between the metanephric mesenchyme and the ureteric bud, the former of which contains nephron progenitors that give rise to glomeruli and renal tubules. Bone morphogenetic protein (BMP) signaling and its modifiers play important roles in many steps of kidney development. BMP4 inhibits ureteric bud attraction, and the BMP antagonist Gremlin is essential for the initial stage of ureteric budding. During mid-gestation, BMP7 maintains the nephron progenitors and, at the same time, sensitizes them to the ureteric bud-derived differentiation signal. Crossveinless2 is a pro-BMP factor, and its absence leads to kidney hypoplasia. After birth, when nephron progenitors have disappeared, Dullard, a phosphatase that inactivates BMP receptors, keeps BMP signaling at an appropriate level. Deletion of *Dullard* results in excessive BMP signaling and apoptosis of the postnatal nephrons. In this review I discuss the similarities and differences of BMP functions in kidney development, as well as in diseases.

Keywords Kidney development · Dullard · Bone morphogenetic proteins · Metanephric mesenchyme

Introduction

A mammalian kidney contains approximately one million nephrons, which are functional units consisting of glomeruli, proximal tubules, loop of Henle, distal tubules, and collecting ducts. During development, the nephron structure is formed by reciprocally inductive interactions between two precursor tissues, the metanephric mesenchyme and the ureteric bud [1]. The former contains multipotent nephron progenitors that express two transcription factors, Six2 and Cited1 [2, 3]. A lineage-tracing study demonstrated that the Six2- or Cited1-expressing mesenchyme gives rise to most parts of the nephron epithelia, including the glomerular podocytes, parietal cells of Bowman's capsule, and tubular cell lineages (proximal tubules, loop of Henle, and distal tubules), but not the collecting duct cells. The collecting duct epithelia derive from the ureteric bud, which expresses *Hoxb7* [4]. These nephron-forming processes start at embryonic day (E) 10.5 and finish within a few days after birth in mice [5]. After this period, the nephron progenitors disappear and no more new nephrons are formed, although the kidney continues to grow until adulthood.

Bone morphogenetic proteins (BMPs) play important roles in various aspects of embryonic development, and BMP4 and BMP7 are the major ligands involved in kidney formation [6]. Their receptors consist of two subtypes, type I and type II, both of which are transmembrane type serine/threonine kinases. Upon ligand binding, the type II receptor phosphorylates itself, as well as the type I receptor. In turn, the type I receptor phosphorylates Smad1/5/8, which is translocated into the nucleus and activates the transcription of target genes. The type I receptor also activates the mitogen-activated protein kinase (MAPK) cascade through Tak. Tak phosphorylates p38 and Jnk, which in turn phosphorylate transcription factors such as c-Jun, Ets1, and Atf2.

BMP4 and its antagonist Gremlin regulate ureteric budding

The combination of kidney and ureter defects caused by gene mutations is termed congenital anomalies of the kidney and urinary tract (CAKUT), and constitutes a major cause of renal failure in the perinatal period in humans [7]. *Bmp4* is

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Histone Lysine-specific Demethylase 1 (LSD1) Protein Is Involved in Sal-like Protein 4 (SALL4)-mediated Transcriptional Repression in Hematopoietic Stem Cells*

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Background: SALL4 plays important roles in regulating the growth of hematopoietic progenitor cells.
Results: SALL4 dynamically recruits histone demethylase LSD1 to specific target genes. LSD1 negatively regulates SALL4-mediated gene expression by affecting local chromatin structure.
Conclusion: Multiple epigenetic modifiers cooperatively modulate SALL4-mediated gene repression.
Significance: This report provides a novel mechanism by which stem cell gene *SALL4* controls hematopoietic progenitor cell properties.

The stem cell protein SALL4 plays a critical role in hematopoiesis by regulating the cell fate. In primitive hematopoietic precursors, it activates or represses important genes via recruitment of various epigenetic factors such as DNA methyltransferases, and histone deacylases. Here, we demonstrate that LSD1, a histone lysine demethylase, also participates in the trans-repressive effects of SALL4. Based on luciferase assays, the amine oxidase domain of LSD1 is important in suppressing SALL4-mediated reporter transcription. In freshly isolated adult mouse bone marrows, both SALL4 and LSD1 proteins are preferentially expressed in undifferentiated progenitor cells and co-localize in the nuclei. Further sequential chromatin immunoprecipitation assay confirmed that these two factors share the same binding sites at the promoter regions of important hematopoietic regulatory genes including *EBF1*, *GATA1*, and *TNF*. In addition, studies from both gain- and loss-of-function models revealed that SALL4 dynamically controls the binding levels of LSD1, which is accompanied by a reversely changed histone 3 dimethylated lysine 4 at the same promoter regions. Finally, shRNA-mediated knockdown of *LSD1* in hematopoietic precursor cells resulted in altered SALL4 downstream gene expression and increased cellular activity. Thus, our data revealed that histone demethylase LSD1 may negatively regulate SALL4-mediated transcription, and the dynamic regulation of SALL4-associated epigenetic factors cooperatively modulates early hematopoietic precursor proliferation.

SALL4 is a zinc-finger transcription factor and is essential for human embryonic development (1–3). We and others have earlier reported that SALL4 plays important roles in maintaining the properties of embryonic stem cells by interacting with Oct4 and Nanog (4–7). Moreover, *SALL4* is one of the few genes that are also involved in tissue stem cell self-renewal and multipotency maintenance (8–10). In isolated mouse bone marrow (BM)[3] $Lin^-/Sca1^+/ckit^+$ (LSK) cells, forced expression of SALL4 dramatically activates multiple hematopoietic stem and progenitor cell (HSPC) regulatory genes including HoxB and Notch factors and leads to a rapid *ex vivo* HSPC expansion, as well as increased cell repopulating abilities *in vivo* (10, 11). More strikingly, by using the *SALL4* transduction methodology, the HSPCs obtained from human peripheral blood are capable of rapid and efficient *ex vivo* expansion by >10,000-fold in the presence of appropriate cytokines (12). These findings provide a novel avenue for achieving clinically significant expansion of human HSPCs. We have sought to examine the potential transcriptional and/or epigenetic mechanisms underlining the observed SALL4 effects on BM progenitor cells. To this end, we and others have reported that SALL4 can silence lineage differentiation genes and modulate cell proliferation by recruiting epigenetic regulators, including DNA methyltransferases (DNMT1, 3A, 3B, 3L) and histone deacetylases (HDAC1 and HDAC2), to target genes (13–15). In addition, SALL4-mediated activation of *Bmi-1*, another important hematopoietic stem cell gene, involves methylation of lysine 4 of histone H3

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[3] The abbreviations used are: BM, bone marrow; CFU, colony-forming unit; BFU-E, burst-forming unit-erythroid; co-IP, co-immunoprecipitation; DNMT, DNA methyltransferase; H3K4, lysine 4 of histone 3; HDAC, histone deacetylase; HSPC, hematopoietic stem and progenitor cell; LSD1, lysine-specific demethylase 1; NuRD, nucleosome remodeling and histone deacetylase; PCPA, trans-2-phynylcyclopropylamine hydrochloride; qPCR, quantitative PCR; qRT-PCR, quantitative RT-PCR.

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Article

Homeoproteins Six1 and Six4 Regulate Male Sex Determination and Mouse Gonadal Development

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SUMMARY

The Y-linked gene *Sry* regulates mammalian sex determination in bipotential embryonic gonads. Here, we report that the transcription factors Six1 and Six4 are required for male gonadal differentiation. Loss of Six1 and Six4 together, but neither alone, resulted in a male-to-female sex-reversal phenotype in XY mutant gonads accompanied by a failure in *Sry* activation. Decreased gonadal precursor cell formation at the onset of *Sry* expression and a gonadal size reduction in both sexes were also found in mutant embryos. Forced *Sry* transgene expression in XY mutant gonads rescued testicular development but not the initial disruption to precursor growth. Furthermore, we identified two downstream targets of Six1/Six4 in gonadal development, *Fog2* (*Zfpm2*) and *Nr5a1* (*Ad4BP/Sf1*). These two distinct Six1/Six4-regulated pathways are considered to be crucial for gonadal development. The regulation of *Fog2* induces *Sry* expression in male sex determination, and the regulation of *Nr5a1* in gonadal precursor formation determines gonadal size.

INTRODUCTION

During mammalian sex determination, expression of the Y-linked gene *Sry* (sex determination region on Y chromosome) shifts the bipotential embryonic gonad toward a testicular fate (Sinclair et al., 1990; Koopman et al., 1991). The fate of the embryonic gonad further determines the sex of an individual and of germ cells.

The genital primordium, the genital ridge, forms on the ventral surface of the mesonephros as paired thickenings of the epithelial layer at around embryonic day (E) 9.5 in mice. This is accompanied by a proliferation of the coelomic epithelium, the precursor of somatic lineages of the gonad that specifically expresses orphan nuclear receptor adrenal 4 binding protein (Ad4BP) (also known as nuclear receptor subfamily 5, group A, member 1 [Nr5a1] or steroidogenic factor1 [Sf1]). Some Nr5a1-positive daughter cells further express *Sry* to become Sertoli cell precursors by E10.5. Soon after *Sry* is expressed, *Sry-related HMG box 9* (*Sox9*) is upregulated in the Sertoli cell precursors (Albrecht and Eicher, 2001; Bullejos and Koopman, 2001; Hatano et al., 1996; Schmahl et al., 2000; Sekido et al., 2004; Sekido and Lovell-Badge, 2007). Transgenic analysis has demonstrated that the expression of either *Sry* or *Sox9* in the bipotential gonad is sufficient to induce the male developmental program (Koopman et al., 1991; Vidal et al., 2001).

Despite the identification of *Sry* as the testis-determining gene in mammals, genetic interactions controlling the earliest steps of male sex determination remain poorly understood. There is little molecular information about the regulation of *Sry* in vivo: genetic inactivation of friend of GATA2 (*Fog2*; also known as zinc finger protein, multitype 2, *Zfpm2*); *Gata4*^{ki}, which abrogates the interaction of Gata4 with Fog; polycomb group gene *M33* (also known as *Cbx2*); and Wilms tumor 1 homolog (*Wt1*) +*KTS* (isoform with lysine, threonine, and serine) resulted in reduced *Sry* expression and a sex-reversal phenotype (Tevosian et al., 2002; Katoh-Fukui et al., 1998; Hammes et al., 2001). In vitro biochemical analyses have demonstrated that WT1, NR5A1, SOX9, GATA4, and SP1 can bind to and transactivate human or pig *SRY* promoters (de Santa Barbara et al., 2000; Hossain and Saunders, 2001; Shimamura et al., 1997; Pilon et al., 2003; Miyamoto et al., 2008; Desclozeaux et al., 1998). Mutant mouse analyses have also revealed that *Nr5a1*, LIM homeobox 9 (*Lhx9*), empty spiracles homeobox 2 (*Emx2*), and *Wt1-KTS* (absence of lysine, threonine, and serine) are essential for the formation and development of the bipotential gonadal primordium (Luo et al., 1994; Shinoda et al., 1995; Sadovsky et al., 1995; Birk et al., 2000; Miyamoto et al., 1997; Wilhelm and Englert, 2002).

The *Six1* and *Six4* genes belong to the mammalian homolog of the *Drosophila sine oculis homeobox* (*Six*) family, including six member genes (*Six1–Six6*) in the mouse genome. These genes encode transcriptional factors with characteristic Six domains and homeodomains (Kawakami et al., 2000). Six1 and Six4 are reported to bind to the MEF3 site (consensus sequence, TCAGGN/t) and transactivate myogenic regulatory factor family genes such as *myogenin* and *Myf5* (Parmacek et al., 1994; Spitz

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Sall4 Is Transiently Expressed in the Caudal Wolffian Duct and the Ureteric Bud, but Dispensable for Kidney Development

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Abstract

The kidney, the metanephros, is formed by reciprocal interactions between the metanephric mesenchyme and the ureteric bud, the latter of which is derived from the Wolffian duct that elongates in the rostral-to-caudal direction. *Sall1* expressed in the metanephric mesenchyme is essential for ureteric bud attraction in kidney development. *Sall4*, another member of the *Sall* gene family, is required for maintenance of embryonic stem cells and establishment of induced pluripotent stem cells, and is thus considered to be one of the stemness genes. *Sall4* is also a causative gene for Okihiro syndrome and is essential for the formation of many organs in both humans and mice. However, its expression and role in kidney development remain unknown, despite the essential role of *Sall1* in the metanephric mesenchyme. Here, we report that mouse Sall4 is expressed transiently in the Wolffian duct-derived lineage, and is nearly complementary to Sall1 expression. While Sall4 expression is excluded from the Wolffian duct at embryonic (E) day 9.5, Sall4 is expressed in the Wolffian duct weakly in the mesonephric region at E10.5 and more abundantly in the caudal metanephric region where ureteric budding occurs. Sall4 expression is highest at E11.5 in the Wolffian duct and ureteric bud, but disappears by E13.5. We further demonstrate that *Sall4* deletion in the Wolffian duct and ureteric bud does not cause any apparent kidney phenotypes. Therefore, Sall4 is expressed transiently in the caudal Wolffian duct and the ureteric bud, but is dispensable for kidney development in mice.

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Introduction

The mammalian kidney, the metanephros, is formed by reciprocally inductive interactions between two precursor tissues, the metanephric mesenchyme and the ureteric bud [1]. The mesenchyme attracts the ureteric bud, while the ureteric bud induces the mesenchyme to differentiate into the epithelia of the glomeruli and renal tubules. Meanwhile, the ureteric buds branch and differentiate into collecting ducts and the ureter. The ureteric bud is derived from the Wolffian duct (nephric duct) that elongates in the rostral-to-caudal direction and reaches the caudal end of the embryo at embryonic (E) day 9.5. At E10.5, the Wolffian duct stimulates the nephrogenic mesenchyme to form mesonephric tubules [2]. The caudal end of the Wolffian duct adjacent to the metanephric mesenchyme bulges, from which ureteric budding occurs at E11.0. Subsequently, the ureteric bud elongates and starts to branch. The ureteric buds branch repetitively until a few days after birth in mice.

spalt (*sal*) was first isolated from *Drosophila* as a region-specific homeotic gene and encodes a nuclear protein characterized by multiple double zinc finger motifs [3]. The *Sall* (sal-like) family is conserved among species, and humans and mice each have four *sal-like* genes (known as *SALL1–4* in humans and *Sall1–4* in mice). Mutations in *SALL1* and *SALL4* have been associated with Townes-Brocks syndrome and Okihiro syndrome, respectively, which are both autosomal dominant diseases that involve abnormalities in various organs, including the ears, limbs, heart, and kidneys [4,5]. Okihiro syndrome is likely to result from *Sall4* haploinsufficiency, because *Sall4* heterozygous mice exhibit similar phenotypes to the human symptoms [6]. *Sall4* is essential for the maintenance of embryonic stem cells [6], and accumulating evidence indicates that *Sall4* is involved in the pluripotency network in

Islet1 Deletion Causes Kidney Agenesis and Hydroureter Resembling CAKUT

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ABSTRACT

Islet1 (Isl1) is a transcription factor transiently expressed in a subset of heart and limb progenitors. During studies of limb development, conditional *Isl1* deletion produced unexpected kidney abnormalities. Here, we studied the renal expression of *Isl1* and whether it has a role in kidney development. *In situ* hybridization revealed *Isl1* expression in the mesenchymal cells surrounding the base of the ureteric bud in mice. Conditional deletion of *Isl1* caused kidney agenesis or hypoplasia and hydroureter, a phenotype resembling human congenital anomalies of the kidney and urinary tract (CAKUT). The absence of *Isl1* led to ectopic branching of the ureteric bud out from the nephric duct or to the formation of accessory buds, both of which could lead to obstruction of the ureter-bladder junction and consequent hydroureter. The abnormal elongation and poor branching of the ureteric buds were the likely causes of the kidney agenesis or hypoplasia. Furthermore, the lack of *Isl1* reduced the expression of *Bmp4*, a gene implicated in the CAKUT-like phenotype, in the metanephric region before ureteric budding. In conclusion, *Isl1* is essential for proper development of the kidney and ureter by repressing the aberrant formation of the ureteric bud. These observations call for further studies to investigate whether *Isl1* may be a causative gene for human CAKUT.

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The mammalian kidney, the metanephros, is formed by reciprocally inductive interactions between two precursor tissues: the metanephric mesenchyme and the ureteric bud. The metanephric mesenchyme attracts the ureteric bud tips toward the mesenchyme and subsequently induces branching of the ureteric buds. The attracted ureteric bud tips in turn induce the mesenchymal cells to differentiate into the epithelia of the glomeruli and renal tubules. Impairment of these processes can lead to a variety of abnormal developmental disorders of the kidney. Meanwhile, the stalks of the ureteric buds elongate and differentiate into collecting ducts and the ureter. The ureteric epithelium also interacts with the surrounding ureteral mesenchyme, which differentiates into the smooth muscle layer of the ureter that pushes the urine downward by peristaltic movements. Therefore, mechanical obstruction of the ureter or malformation of the smooth muscle layer causes dilatation of the ureter (hydroureter) and, in more severe cases, of the renal pelvis (hydronephrosis). The combination of kidney and ureter defects caused by gene mutations is termed congenital anomalies of the kidney and urinary tract (CAKUT) and

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Dullard/Ctdnep1 Modulates WNT Signalling Activity for the Formation of Primordial Germ Cells in the Mouse Embryo

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Abstract

Dullard/Ctdnep1 is a member of the serine/threonine phosphatase family of the C-terminal domain of eukaryotic RNA polymerase II. Embryos lacking Dullard activity fail to form primordial germ cells (PGCs). In the mouse, the formation of PGCs is influenced by BMP4 and WNT3 activity. Although Dullard is reputed to negatively regulate BMP receptor function, in this study we found mutations in *Dullard* had no detectable effect on BMP4 and p-Smad activity. Furthermore *Dullard* mutations did not influence the dosage-dependent inductive effect of *Bmp4* in PGC formation. However, *Dullard* may function as a positive regulator of WNT signalling. Combined loss of one copy each of *Dullard* and *Wnt3* had a synergistic effect on the reduction of PGC numbers in the compound heterozygous embryo. In addition, loss of Dullard function was accompanied by down-regulation of WNT/β-catenin signalling activity and a reduction in the level of Dishevelled 2 (Dvl2). Therefore, Dullard may play a role in the fine-tuning of WNT signalling activity by modulating the expression of ligands/antagonists and the availability of Dvl2 protein during specification of the germ cell lineage.

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Introduction

Specification of the germ cell lineage in the mouse begins with activation of *Prdm1* (*Blimp1*) in a subset of epiblast cells in the proximal region of the pre-gastrulation mouse embryo [1]. Analysis of germ cell formation in mutant mouse embryos has revealed the critical role of bone morphogenetic protein (BMP) signalling in the induction of primordial germ cell (PGC) precursors. The PGC population is lost or greatly reduced in embryos that are deficient for BMP activity, which is caused by losses of *Bmp4* and *Bmp8b* in the extraembryonic ectoderm, and *Bmp2* and *Alk2* (encoding a Type I BMP receptor) in the visceral endoderm, or *Smad1*, *4* and *5* (signal transduction factors) in the embryo [2–12]. In addition, WNT signalling plays a role in PGC formation. PGCs are absent in embryos lacking *Wnt3* activity and WNT3A is required for priming epiblast cells to respond to induction by BMP4 to differentiate into PGCs [13].

Dullard (also known as *C-terminal domain nuclear envelope phosphatase 1; Ctdnep1*) was identified as a gene that is expressed in the pronephros and neural tissues of *Xenopus laevis* embryos [14]. *Dullard* encodes a protein serine/threonine phosphatase with a characteristic catalytic motif, DXDX(T/V). It is a member of an emerging family of phosphatases that dephosphorylate target substrates [15,16]. This family is also known as the phosphatase family of the C-terminal domain (CTD) of eukaryotic RNA polymerase II (polII), which dynamically regulates transcription by recruiting different factors to mRNA through its multiple phosphorylation activities [17]. Other SCP/transcription factor IIF-interacting CTD phosphatases that are closely related to Dullard, e.g. small CTD phosphatases (SCPs), play a role in modulation of the expression level of specific genes. Such phosphatases silence neuronal genes in non-neuronal cells to suppress inappropriate neuronal gene expression during cell fate decision. This regulatory activity is mediated through an interaction with the repressor element 1-silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) complex [18]. Bioinformatic analyses of human DULLARD has revealed that the protein contains two potential membrane-spanning regions in the N-terminal, which may direct the localization of DULLARD to the nuclear envelope, where it dephosphorylates a nuclear membrane-associated phosphatidic acid phosphatase in

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The phosphatase Dullard negatively regulates BMP signalling and is essential for nephron maintenance after birth

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Most kidney nephron components, including glomeruli and renal tubules, derive from the metanephric mesenchyme. The overall differentiation into each component finishes at birth, but the molecular events linking the perinatal and adult kidneys remain elusive. *Dullard* was cloned from *Xenopus* kidneys, and encodes a phosphatase that negatively regulates BMP signalling. Here we report that *Dullard* deletion in the murine metanephric mesenchyme leads to failure of nephron maintenance after birth, resulting in lethality before adulthood. The nephron components are lost by massive apoptosis within 3 weeks after birth, leading to formation of a large hollow with a thin-layered cortex and medulla. Phosphorylated Smad1/5/8 is upregulated in the mutant nephrons, probably through cell-autonomous inhibitory effects of Dullard on BMP signalling. Importantly, administration of the BMP receptor kinase inhibitor LDN-193189 partially rescued the defects caused by *Dullard* deletion. Thus, *Dullard* keeps BMP signalling at an appropriate level, which is required for nephron maintenance in the postnatal period.

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Different assemblies of Notch receptors coordinate the distribution of the major bronchial Clara, ciliated and neuroendocrine cells

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SUMMARY

In the developing lung, it is thought that the terminal buds of elongating airways contain a population of multipotent epithelial progenitors. As the bronchial tree extends, descendants of these cells give rise to lineage-restricted progenitors in the conducting airways via Notch signaling, which is involved in the establishment of epithelial Clara, ciliated and pulmonary neuroendocrine (NE) cell populations. However, the precise molecular details of this selection process are still emerging. Our stepwise removal of the three Notch receptors from the developing lung epithelium reveals that, whereas Notch2 mediates the Clara/ciliated cell fate decision with negligible contributions from Notch1 and Notch3, all three Notch receptors contribute in an additive manner to regulate the abundance of NE cells and the size of the presumptive pulmonary neuroepithelial body (pNEB) as a result of mutual interactions between NE cells and the Notch-dependent, SSEA-1$^+$, CC10$^-$ cell population surrounding the pNEB (SPNC cells). Ectopic expression of the Notch1 or Notch2 intracellular domain was sufficient to induce SSEA-1$^+$ cells and to suppress pNEB formation without expending Clara cells. We provide evidence that the additive functions of Notch receptors, together with other signaling pathways, maintains the expression of Hes1, a key regulator of NE cell fate, and that maintenance of Hes1 expression in epithelial cells is key to the regulation of pNEB size. These results suggest that two different assemblies of Notch receptors coordinate the numbers and distribution of the major epithelial cell types in the conducting airway during lung organogenesis.

KEY WORDS: Lung, Notch signaling, Neuroendocrine, Mouse

INTRODUCTION

Lung development relies on reciprocal mesenchymal-epithelial interactions orchestrated by temporal and spatial expression waves of multiple secreted factors and their downstream effectors (Morrisey and Hogan, 2010). Airway branching morphogenesis takes place at E11.5-16.5 in the mouse (the pseudoglandular stage). The terminal buds contain a population of multipotent epithelial progenitors during this period, which give rise to lineage-restricted descendants that produce at least seven major cell types in the 'stalk' region (Perl et al., 2002b; Rawlins et al., 2009). Thus, the early stalks form the proximal airway, then the distal airways and finally the alveoli (Cardoso and Lü, 2006). The Clara, ciliated and pulmonary neuroendocrine (NE) cells are common throughout the conducting airways, but their ratios vary along the proximodistal axis. In the murine tracheal epithelium, the basal cell generates goblet cells, a few Clara cells and many ciliated cells (Hong et al., 2004; Rock et al., 2009). In the distal bronchioles, Clara cells are more abundant than ciliated cells; a few NE cell clusters (called neuroepithelial bodies, or NEBs) per unit area are also present. The distal-most alveoli are lined with a single thin layer of flat type I cells and cuboidal type II cells (Morrisey and Hogan, 2010).

The Notch signaling pathway plays an important role in the developing respiratory system. Notch genes encode single-transmembrane receptors that mediate short-range communication between cells in all animal species (Kopan and Ilagan, 2009). When Notch binds to its ligand expressed on adjacent cells, a negative control region unfolds that permits the shedding of its extracellular domain by ADAM proteases. A subsequent cleavage within the transmembrane domain executed by the enzyme γ-secretase (Mumm and Kopan, 2000; Kopan and Ilagan, 2009) releases the Notch intracellular domain (NICD). NICD translocates to the nucleus, where it associates with Rbpj/Cbf-1, a DNA-binding protein. The NICD/Rbpj complex recruits the adaptor mastermind and assembles a transcription activation complex on target promoters (Lubman et al., 2004). In mammals, four Notch homologs (*Notch1* to *Notch4*) and at least five ligands (*Jag1*, *Jag2*, *Dll1*, *Dll3* and *Dll4*) mediate these signaling events. There are many auxiliary factors that modulate Notch signaling. One of these, protein *O*-fucosyltransferase 1 (Pofut1), conjugates *O*-fucose to specific extracellular EGF repeats, modifications that are essential for productive Notch-ligand interactions in mammals (Okamura and Saga, 2008; Stahl et al., 2008). Although Pofut1 may have additional substrates, *Pofut1*-null mice display Notch signaling defects that are similar to those associated with the loss of γ-secretase or Rbpj (Shi and Stanley, 2003; Okamura and Saga, 2008).

Conditional removal of Pofut1 or Rbpj from lung bud epithelia promoted ciliated cell expansion at the expense of Clara cells (Tsao et al., 2009; Morimoto et al., 2010). By contrast, constitutive

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Phosphorylation of Kif26b Promotes Its Polyubiquitination and Subsequent Proteasomal Degradation during Kidney Development

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Abstract

Kif26b, a member of the kinesin superfamily proteins (KIFs), is essential for kidney development. *Kif26b* expression is restricted to the metanephric mesenchyme, and its transcription is regulated by a zinc finger transcriptional regulator Sall1. However, the mechanism(s) by which Kif26b protein is regulated remain unknown. Here, we demonstrate phosphorylation and subsequent polyubiquitination of Kif26b in the developing kidney. We find that Kif26b interacts with an E3 ubiquitin ligase, neural precursor cell expressed developmentally down-regulated protein 4 (Nedd4) in developing kidney. Phosphorylation of Kif26b at Thr-1859 and Ser-1962 by the cyclin-dependent kinases (CDKs) enhances the interaction of Kif26b with Nedd4. Nedd4 polyubiquitinates Kif26b and thereby promotes degradation of Kif26b via the ubiquitin-proteasome pathway. Furthermore, Kif26b lacks ATPase activity but does associate with microtubules. Nocodazole treatment not only disrupts the localization of Kif26b to microtubules but also promotes phosphorylation and polyubiquitination of Kif26b. These results suggest that the function of Kif26b is microtubule-based and that Kif26b degradation in the metanephric mesenchyme via the ubiquitin-proteasome pathway may be important for proper kidney development.

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Introduction

The kidney is composed of minimum units called nephrons, which maintain an appropriate homeostatic balance of water and salt levels and remove nitrogenous metabolic waste products. The tubular epithelial network of the nephron originates from two different tissues, the ureteric bud and the metanephric mesenchyme, that form the collecting duct system and the renal tubules, respectively [1,2]. The signals from the metanephric mesenchyme, such as glial cell line-derived neurotrophic factor (GDNF), induce sprouting of the ureteric bud from the caudal region of the Wolffian duct and invasion of the ureteric buds into the metanephric mesenchyme. Wnt9b secreted from the ureteric buds induces Wnt4 expression in the mesenchyme [3], and Wnt4 induces the pre-tubular aggregates of the condensed mesenchyme beneath the ureteric bud tips to form renal vesicles in a cell-autonomous manner [4]. Renal vesicles differentiate into each segment of the nephron, including the glomerulus, proximal tubule, loop of Henle, and distal tubule, to eventually form functional nephrons [1,2].

The kinesin superfamily proteins (KIFs) are known to be important molecular motors that are involved in the microtubule- and ATP-dependent transport of various cargos, including membranous organelles, protein complexes, and mRNAs, to specific destinations [5]. Accumulating evidence demonstrates the importance of KIFs in the regulation of many physiological events, including higher brain function, tumor suppression, and developmental patterning. Kif26b was originally identified by a database search of the mouse genome for DNA sequences that contained a motif similar to the kinesin motor domain [6]. Kif26b is classified to the Kinesin-11 family along with Kif26a, an unconventional kinesin that lacks microtubule-based motility [7]. Human KIF26A does not contain the conserved amino acid sequences that are required for motor activity but retains the microtubule-associating ability as well as other conserved KIFs. Smy1p, a Kinesin-11 family member from *Saccharomyces cerevisiae*, is thought not to be motile, especially along microtubules, due to the deviance in both a catalytic pocket for ATP hydrolysis and the microtubule-binding sites [8]. These reports suggest that Kif26b also does not function as a microtubule-based motor. Therefore, the biochemical and cellular functions of Kif26b remain to be clarified.

We recently reported that *Kif26b*-knockout mice exhibit kidney agenesis or hypoplasia [9]. In *Kif26b*-null embryos, the ureteric buds elongate and migrate in proximity to, but do not invade, the

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Stem Cells, Tissue Engineering, and Hematopoietic Elements

Generation of Kidney from Pluripotent Stem Cells via Blastocyst Complementation

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Because a shortage of donor organs has been a major obstacle to the expansion of organ transplantation programs, the generation of transplantable organs is among the ultimate goals of regenerative medicine. However, the complex cellular interactions among and within tissues that are required for organogenesis are difficult to recapitulate *in vitro*. As an alternative, we used blastocyst complementation to generate pluripotent stem cell (PSC)–derived donor organs *in vivo*. We hypothesized that if we injected PSCs into blastocysts obtained from mutant mice in which the development of a certain organ was precluded by genetic manipulation, thereby leaving a niche for organ development, the PSC-derived cells would developmentally compensate for the defect and form the missing organ. In our previous work, we showed proof-of-principle findings of pancreas generation by injection of PSCs into pancreas-deficient *Pdx1*$^{-/-}$ mouse blastocysts. In this study, we have extended this technique to kidney generation using *Sall1*$^{-/-}$ mouse blastocysts. As a result, the defective cells were totally replaced, and the kidneys were entirely formed by the injected mouse PSC-derived cells, except for structures not under the influence of *Sall1* expression (ie, collecting ducts and microvasculature). These findings indicate that blastocyst complementation can be extended to generate PSC-derived kidneys. This system may therefore provide novel insights into renal organogenesis. *(Am J Pathol 2012, 180:2417–2426; http://dx.doi.org/10.1016/j.ajpath.2012.03.007)*

At present, most patients with end-stage renal failure are treated by dialysis. Some patients eventually undergo kidney transplantation, but this option is limited by a shortage of donor organs. Therefore, the number of patients undergoing dialysis continues to grow, with complications, poor quality of life, and increasing medical costs. Moreover, the shortage of donor organs has led to other social problems, such as organ trafficking, transplant tourism, and transplant commercialism.[1] Under these circumstances, to transplant donor organs derived from pluripotent stem cells would be a much welcomed alternative. Induced-PSC (iPSC) technology[2,3] has recently enabled the generation of individual- or patient-derived PSCs, with studies of disease-targeting stem cell replacement therapy. However, the generation of an organ from iPSCs is considered impractical because it remains difficult to replicate *in vitro* the complex interactions among cells and tissues during organogenesis. To overcome this obstacle, we attempted to generate organs *in vivo* using the blastocyst complementation technique originally reported by Chen et al[4] in analyses of genes involved in lymphocyte development. We reported the successful application of this technique to generate PSC-derived mouse and rat pancreas in the *Pdx1*$^{-/-}$ mouse.[5] In the PSC-generated pancreas, defective cells were totally replaced, and the pancreas was formed almost entirely by the injected mouse and rat PSC-derived cells. The mouse and rat PSC-derived pancreas produced a variety of hormones, including insulin, and the transplantation of PSC-derived pancreas islets improved hyperglycemia in a diabetic mouse model. The premise driving this work is that a niche for organogenesis can be created in postblastocyst mutant mouse embryos that are genetically precluded from developing a particular or-

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Cell Stem Cell
Article

Functional Antagonism between Sall4 and Plzf Defines Germline Progenitors

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SUMMARY

Transcription factors required for formation of embryonic tissues often maintain their expression in adult stem cell populations, but whether their function remains equivalent is not clear. Here we demonstrate critical and distinct roles for Sall4 in development of embryonic germ cells and differentiation of postnatal spermatogonial progenitor cells (SPCs). In differentiating SPCs, Sall4 levels transiently increase and Sall4 physically interacts with Plzf, a transcription factor exclusively required for adult stem cell maintenance. Mechanistically, Sall4 sequesters Plzf to noncognate chromatin domains to induce expression of Kit, a target of Plzf-mediated repression required for differentiation. Plzf in turn antagonizes Sall4 function by displacing Sall4 from cognate chromatin to induce Sall1 expression. Taken together, these data suggest that transcription factors required for embryonic tissue development postnatally take on distinct roles through interaction with opposing factors, which hence define properties of the adult stem cell compartment.

INTRODUCTION

The formation of distinct tissues in the developing embryo is dependent on a hierarchy of developmental decisions. A critical aspect of the formation of multiple tissues is the establishment of an associated stem cell compartment that supports organ maintenance over the lifespan of the animal. It has been suggested that tissue-specific stem cells in the adult maintain characteristics of the embryonic rudiment that gave rise to the tissue during development (Slack, 2008). Indeed, key transcription factors necessary for formation of the embryonic tissue are often expressed in the established adult stem cell pool and regulate its activity (Slack, 2008). However, embryonic cells are by nature highly dynamic, contrasting with the more stable and long-lived properties of adult stem cells. Thus it is unclear how embryonic factors would operate in adult stem cell compartments.

In adult testis, there is a pool of germline stem/progenitor cells that have self-renewal potential and continuously generate differentiating daughter cells for the subsequent production of haploid spermatozoa. In mice, this cell population is composed of isolated A_s spermatogonia together with cysts of interconnected A_{pr} and A_{al} cells, collectively referred to as undifferentiated spermatogonia or spermatogonial progenitor cells (SPCs) (de Rooij and Russell, 2000; Hobbs et al., 2010; Seandel et al., 2007). Traditionally, A_s cells were thought to form the stem cell compartment while A_{pr} and A_{al} represented committed, transit-amplifying cells. However, recent studies indicate that stem cell potential is retained by all SPCs (Simons and Clevers, 2011). Differentiation of SPCs is marked by induction of the receptor tyrosine kinase c-Kit (Schrans-Stassen et al., 1999) and the formation of A_1 spermatogonia, which subsequently undergo a series of rapid mitotic divisions prior to meiosis.

Specification of germ cell fate during mouse embryogenesis initiates in the early postimplantation embryo with the formation of primordial germ cells (PGCs) from the proximal epiblast (Hayashi et al., 2007). PGCs subsequently migrate to the developing gonad where they differentiate into gonocytes (Figure 1A). Female gonocytes enter meiosis prior to birth, while male gonocytes undergo mitotic cell cycle arrest. During the first postnatal week, male gonocytes resume proliferation and migrate from the seminiferous tubule lumen to the basement membrane where they directly generate both differentiating spermatogonia for the first round of spermatogenesis plus a pool of cells with SPC properties (Figure 1A) (Yoshida et al., 2006). Germline stem cell activity is largely attained during this early period of postnatal testis development (McLean et al., 2003; Shinohara et al., 2001) and few functional differences exist between this nascent SPC pool and that of the adult (Ebata et al., 2007).

SPC maintenance is dependent on their expression of the POZ-*Krüppel* family transcription factor *Promyelocytic Leukemia Zinc Finger* (*Plzf*), because mice lacking *Plzf* show progressive loss of these cells, resulting in sterility (Buaas et al., 2004; Costoya et al., 2004). Importantly, formation of the germ cell

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RESEARCH ARTICLE

Sall1 regulates cortical neurogenesis and laminar fate specification in mice: implications for neural abnormalities in Townes-Brocks syndrome

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SUMMARY

Progenitor cells in the cerebral cortex undergo dynamic cellular and molecular changes during development. Sall1 is a putative transcription factor that is highly expressed in progenitor cells during development. In humans, the autosomal dominant developmental disorder Townes-Brocks syndrome (TBS) is associated with mutations of the *SALL1* gene. TBS is characterized by renal, anal, limb and auditory abnormalities. Although neural deficits have not been recognized as a diagnostic characteristic of the disease, ~10% of patients exhibit neural or behavioral abnormalities. We demonstrate that, in addition to being expressed in peripheral organs, Sall1 is robustly expressed in progenitor cells of the central nervous system in mice. Both classical- and conditional-knockout mouse studies indicate that the cerebral cortex is particularly sensitive to loss of Sall1. In the absence of Sall1, both the surface area and depth of the cerebral cortex were decreased at embryonic day 18.5 (E18.5). These deficiencies are associated with changes in progenitor cell properties during development. In early cortical progenitor cells, Sall1 promotes proliferative over neurogenic division, whereas, at later developmental stages, Sall1 regulates the production and differentiation of intermediate progenitor cells. Furthermore, Sall1 influences the temporal specification of cortical laminae. These findings present novel insights into the function of Sall1 in the developing mouse cortex and provide avenues for future research into potential neural deficits in individuals with TBS.

INTRODUCTION

The mature cortex is a six-layered structure that controls complex functions, including motor coordination, and auditory, visual and somatosensory processing, as well as cognition (reviewed in Rosner, 1970). Appropriate regulation of cell number, cell-type specification, laminar positioning and circuit formation is essential for normal functioning of the mature nervous system. Dysregulation of cortical development can lead to a variety of gross cortical malformations and psychiatric disorders, including lissencephaly, periventricular heterotopia, microcephaly, epilepsy, autism and schizophrenia (reviewed in Arnold, 1999; Lian and Sheen, 2006; Pilz et al., 2002; Polleux and Lauder, 2004; Schwartzkroin and Walsh, 2000).

The type of division a progenitor cell (PC) makes is an important mechanism in regulating cell number and fate in the cortex. Early in development, the PC population expands by symmetric divisions, resulting in the production of two progeny radial glial cells (RGCs) (Noctor et al., 2008; Takahashi et al., 1996b). At the onset of neurogenesis (~E10.5 in mice), RGC asymmetric neurogenic divisions result in the generation of a neuroblast and an RGC (Haubensak et al., 2004; Noctor et al., 2001; Noctor et al., 2008). By mid-neurogenesis (~E14.5 in mice) these divisions represent the predominant division type in the ventricular zone (VZ) (Noctor et al., 2004). Subsequent asymmetric RGC divisions produce an RGC and an intermediate progenitor cell (IPC) (Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004; Noctor et al., 2008). IPCs (also referred to as basal progenitors) predominately undergo symmetric terminal neurogenic division at the basal side of the VZ or within the subventricular zone (SVZ), resulting in the production of two neurons (Attardo et al., 2008; Haubensak et al., 2004; Miyata et al., 2004; Noctor et al., 2004; Noctor et al., 2008). Although rare, symmetric proliferative IPC divisions have also been reported, resulting in the production of two daughter IPCs (Miyata et al., 2004; Noctor et al., 2004). Recent studies suggest that IPCs give rise to the majority of cortical neurons, so perturbing this population during development has the potential to impact neuronal organization and ultimately behavior (Haubensak et al., 2004; Martinez-Cerdeno et al., 2006; Miyata et al., 2004; Noctor et al., 2004; Noctor et al., 2008; Pontious et al., 2008; Sessa et al., 2008). The molecular mechanisms regulating specification, maintenance and fate of this population are just beginning to be understood.

This study investigated the role of a member of the Sall gene family, *Sall1*, in the developing brain and identifies a unique role for the *Sall1* gene in regulating PCs in the cerebral cortex. Sall1 is a C_2H_2 zinc-finger-containing putative transcription factor that is highly expressed in the developing CNS and peripheral organs. Previous studies have shown that members of the Sall gene family play a role in cell cycle regulation, proliferation, neuronal differentiation, migration and cell adhesion in other species (Barembaum and Bronner-Fraser, 2004; Basson and Horvitz, 1996;

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