Defining the Signals that Constitute the Nephron Progenitor Niche

Thomas J. Carroll and Amrita Das

Departments of Internal Medicine (Nephrology) and Molecular Biology, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas

ABSTRACT

For decades we have known that reciprocal inductive interactions between the embryonic ureteric bud and the metanephric mesenchyme are the basis for kidney development. Signals from the mesenchyme promote the branching of the bud, whereas signals from the bud regulate the survival, proliferation, and differentiation of nephron progenitors. Due to the complex nature of the bud-derived signals, progress in identifying these factors has been slow. However, in the last several years, tremendous advances have been made in identifying specific roles for various secreted proteins in nephron progenitor cell development. Here, we briefly review the roles for Fgfs and Wnts in induction of the nephron progenitors.


The mammalian kidney is an amazingly complex organ composed of multiple cell types including epithelia, vasculature, fibroblasts, and nerves. Remarkably, this complexity arises from relative simplicity. At the earliest stages of renal development, the nascent organ consists of two distinct tissues: an epithelial structure, known as the ureteric bud, surrounded by a cap of mesenchyme known as the metanephric mesenchyme (MM). The majority of the cells found in the adult organ will arise from progenitor cells located within these two tissues, including the vasculature.

During development, the ureteric bud undergoes repeated branching morphogenesis within the mesenchyme, eventually forming the entire collecting duct system and the ureter. The mesenchyme is a heterogeneous population of cells from at least two distinct (and probably three) cellular lineages that give rise to the proximal portion of the nephron, the Bowman’s capsule through the connecting tubule, the mural cells, and possibly the microvasculature and renal nerves.

The mesenchymal cells that give rise to the proximal nephron are referred to as the nephron progenitors or cap mesenchyme. The nephron progenitor cells undergo a mesenchymal-to-epithelial transition (MET) to form an epithelial tubule referred to as a renal vesicle. This spherical structure subsequently undergoes significant growth and morphogenesis to form the mature nephron.

Embryologic studies performed 50 years ago determined that reciprocal inductive interactions between the MM and the ureteric bud mediate the development of the kidney. Multiple distinct signals from the MM promote ureteric bud branching. In a reciprocal manner, the ureteric bud produces signals that stimulate the survival, proliferation, and MET of the MM. A delicate balance is struck between these processes ensuring proper nephron endowment.

For years, cell biologists have sought to identify the factors responsible for the inductive interactions that drive kidney development. Because many of these processes are likely interdependent, it is in some cases difficult to assign primary versus secondary roles; can proliferation be induced without first inducing survival? Here we discuss our current understanding of the molecules emanating from the ureteric bud that regulate survival, proliferation, and MET of the nephron progenitor cells (Figure 1). It is important to remember that what we describe is likely an oversimplification and, as the reader may realize, the situation in vivo is likely to be much more complex.

SURVIVAL

Without the ureteric bud, isolated MM undergoes apoptosis within 48 hours. Utilizing a combination of biochemistry and candidate protein approaches, investigators sought out ureteric bud–produced factors that were sufficient to promote survival of isolated MM. The top candidates for this role are members of the fibroblast growth factor (Fgf) family. Fgfs support survival of the nephron progenitors without inducing MET.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Thomas J. Carroll, Departments of Internal Medicine (Nephrology) and Molecular Biology, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75390-9148. Email: Thomas.Carroll@utsouthwestern.edu

Copyright © 2013 by the American Society of Nephrology
Subsequent analysis determined that only two Fgfs (2 and 9) that are expressed in the mouse ureteric bud at the time of induction are also sufficient to promote survival. However, independently, neither of these genes is necessary for survival, suggesting that they may act redundantly. More recent studies have shown that Fgf20 is also sufficient for survival and it is necessary to maintain proper cell number. However, Fgf20 is expressed within the nephron progenitors themselves, not the ureteric bud. Interestingly, coablation of Fgf9 (from the ureteric bud) and Fgf20 leads to complete agenesis and progenitor cell apoptosis, closely matching the phenotype observed upon surgical removal of the ureteric bud. A similar phenotype is observed after progenitor cell–specific removal of the Fgf receptors Fgfr 1 and 2. These data suggest that Fgf signaling is necessary and sufficient to induce survival of the progenitor cells although there are different cellular sources of the ligands. Fgf9 (and possibly 2) from the bud cooperates with Fgf20 within the mesenchyme to induce survival. These findings raise the question as to why the ureteric bud is required for survival if Fgf20 expression in the progenitors alone is sufficient? A bud–derived factor must be required for induction of Fgf20 expression and/or activity within the mesenchyme. This factor could be Fgf2, cooperating with Fgf9, or other unidentified molecules.

The cap mesenchyme shows regionalized gene expression potentially representing progenitors at distinct stages of differentiation. Depending on the source and treatment of the isolated progenitors, Fgfs appear to only maintain survival of cells at a specific stage of differentiation. More work will be required to tease out the specific roles of Fgfs during progenitor cell survival, proliferation, and differentiation.

**MET AND PROLIFERATION**

Wnt9b, a member of a large family of secreted signaling molecules, is expressed in the developing ureteric bud throughout kidney development. Treatment of isolated mesenchyme with Wnt9b leads to survival, proliferation, and MET. Kidneys lacking Wnt9b form a ureteric bud capped by nephron progenitor cells, but fail to undergo MET. Thus, Wnt9b is necessary and sufficient to induce MET.

Although the nephron progenitor population is initially present and specified correctly in Wnt9b mutants, it shows a very low proliferation rate relative to wildtype. Treatment of isolated mesenchyme with Wnt9b leads to expansion and maintenance of the nephron progenitors alongside the MET, suggesting that Wnt9b also promotes proliferation. This could be a direct role for Wnt9b on the progenitors or an indirect role resulting from Wnt9b’s ability to induce renal vesicles. In support of the former, in addition to directly inducing target genes in the subset of progenitors undergoing MET, Wnt9b also directly activates targets within the renewing progenitors. Although there is currently no direct link between Wnt9b progenitor target genes and proliferation, this model fits nicely with previous findings showing that the kinetics of induction of proliferation and MET are nearly identical, indicating that these processes may be regulated by the same factor.

The nephron progenitors survive for several days in the absence of Wnt9b. However, isolated progenitors treated with Wnt9b survive as they proliferate and undergo MET. If Wnt9b is not necessary for survival, then how is it sufficient? The most likely explanation is that Wnt9b triggers activation of the Fgf pathway within the progenitors. It has been shown previously that Wnt9b...
induces the expression of Fgfl8 and 9 within the PTAs. It is plausible that the induction of these factors, perhaps in conjunction with mesenchymal Fgf20, can lead to progenitor survival independent of the ureteric bud. In addition, it appears that the Wnt and Fgfs pathways are codependent in the kidney. Microarray analysis of Wnt9b mutants showed abrogated expression of several canonical Fgf targets, such as Etv5, even though the ureteric bud still expresses Fgf9 and presumably Fgf2 (data not shown). Recent transcriptional profiling of kidneys lacking Fgfr1I, a transmembrane protein necessary for proper Fgf signaling, shows a striking overlap with the Wnt9b mutant profile. Indeed, the Fgfr1I and Wnt9b mutant phenotypes appear nearly identical, strongly supporting the idea that Wnt9b and Fgf signaling interact during kidney development. Determining the precise nature of these interactions will certainly be informative.

DEFINING THE INDUCER

After >50 years of investigation, we have gained great insight into the identity of the factors produced by the ureteric bud that regulate the survival, proliferation, and differentiation of nephron progenitors. Consideration of all of the data suggests a model whereby ureteric bud–derived Fgfs (2 and possibly 9) promote survival, whereas Wnt9b promotes proliferation and differentiation of the mesenchyme. Once nephrogenesis is under way, the induction by the bud continues but is also supplemented by the production of additional factors from the ureteric bud (such as LIF), the pre-tubular aggregates and renal vesicles (LIF, Fgfl8, Fgf9, and Wnt4), and the progenitors themselves (Fgf20). Wnt9b may also be required to make the progenitors competent to respond to the Fgfs and vice versa.

The ultimate goal of kidney progenitor cell research is to generate a self-renewing population of multipotent kidney progenitor cells that can be used in therapeutic contexts. Even with our current knowledge, this goal has not been attained. Clearly there are additional factors required that we must strive to identify and understand. For instance, it is still not clear what makes some cells responding to Wnt9b activate the progenitor cell renewal pathway versus the differentiation pathway. Branching of the ureteric bud is regulated by several signals arising from distinct cell lineages within the mesenchyme. Similarly, it is likely that multiple signals from multiple different cell types act in concert with the Fgfs and Wnts to constitute the nephron progenitor niche.

Finally, several studies show that Bmp7 plays an essential role in the renewal/maintenance of the nephron progenitors in vivo. BMP7 cooperates with Fgfs to promote renewal and prevent differentiation. However, Bmp7 is expressed in both the ureteric bud and the progenitor cells. At this point, it is unclear whether Bmp7 functions in a cell autonomous or nonautonomous manner, or both. Because this review focuses on bud-derived factors, we have neglected this molecule, but it clearly plays an important role and it will be of great interest to further elucidate how Bmp, Fgf, and Wnt signaling networks interact to form the final organ.

Kidney disease is reaching epidemic numbers throughout the world. Unfortunately, therapeutic options are still quite limited. Hopefully, recent advances in our understanding of the biology underlying kidney progenitor cell survival, proliferation, and differentiation will facilitate stem cell therapies in the near future allowing engineering of replacement tissue to finally deliver on the promise of the last 50 years of research.

ACKNOWLEDGMENTS

We apologize to those whose research was neglected due to constraints on the length of this review. We thank Ondine Cleaver for reading and commenting on the manuscript, as well as members of the Carroll laboratory for scientific discussions.

Research in the Carroll laboratory is supported by grants from the National Institutes of Health (1R01DK80004, 5P30DK079328), the American Heart Association, the National Kidney Foundation, and the March of Dimes.

DISCLOSURES

None.

REFERENCES

13. Perantoni AO, Dove LF, Karavanova I: Basic fibroblast growth factor can mediate the early inductive events in renal development.


25. Gerber SD, Steinberg F, Beyeler M, Villiger PM, Trueb B: The murine Fgfr1 receptor is essential for the development of the metanephric kidney. Dev Biol 335: 106–119, 2009


