

Lessons Learned About Adult Kidney Stem Cells From the Malpighian Tubules of *Drosophila*

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ABSTRACT

All multicellular organisms have a specialized organ to concentrate and excrete wastes from the body. The kidneys in vertebrates and the malpighian tubules in *Drosophila* accomplish these functions. Mammals and *Drosophila* share some similar features during renal tubular development. Vertebrate kidneys are derived through the mutual induction of the ureteric bud and metanephric mesoderm, whereas the malpighian tubules of *Drosophila* develop from the hindgut primordium and visceral mesoderm. The vertebrate kidney also has the capacity to recover and regenerate following episodes of acute injury. Previous studies suggest that stem cells and progenitor cells may be involved in the repair and regeneration of injured renal tissue. However, studies differ as to the source of the regenerating renal cells. Recently, multipotent stem cells in *Drosophila* malpighian tubules were identified, and it was demonstrated that several differentiated cells in the malpighian tubules arise from these stem cells. In this article, the current understanding of kidney development and stem cell fate in mammal and *Drosophila* is compared. Furthermore, the potential application of the adult renal stem cells in kidney repair and the treatment of kidney cancers are discussed.

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All multicellular animals concentrate and excrete the waste products of metabolism through specialized excretory organs. The excretory organs vary in different animals but are similar in form and function. In mammals, the kidney is an important excretory and homeostatic organ of metabolism. It performs many essential functions and excretes a wide variety of waste products of metabolism, such as urea and uric acid.¹ The malpighian tubules of *Drosophila* function as the fly kidney, which together with hindgut, acts as an excretory and osmoregulatory system. The major functions of malpighian tubules include the excretion of nitrogen wastes, excess water, and potassium, and organic metabolites, selectively reabsorbing fluid in the proximal tubule of malpighian tubules and hind-

gut and acting as an autonomous immune system.^{2–4} In this review, we compare kidney development and the use of adult kidney stem cells in *Drosophila* and mammals. We also discuss the potential applications of adult kidney stem cells in kidney regeneration after acute injury and in the treatment of kidney cancers.

COMMON ELEMENTS OF KIDNEY DEVELOPMENT

The kidney has become an excellent model organ for understanding epithelial-mesenchymal interactions, epithelial cell polarization, and branching morphogenesis. The mammalian embryonic kidney undergoes three stages of devel-

opment: the transient pronephros, the mesonephros, and metanephros forming the adult kidney. All three embryonic kidneys arise from the intermediate mesoderm (mesomere), a strip of cells that connects the somite (dorsal mesoderm) to the lateral plate mesoderm. The adult metanephros is derived from two sources: the ureteric bud (UB; metanephric diverticulum) and the metanephric mesoderm (MM).⁵ A process of mutual induction between the UB and MM initiates kidney development, in which branching of the UB is dependent on the MM and the differentiation of nephrons is integrated with each new collecting tubule. First, the UB, an epithelial tissue, invades the adjacent MM and induces the surrounding condensed mesenchymal cells to undergo mesenchymal-to-epithelial transition. The condensed mesenchymal cells then induce the UB to branch and form new ureteric tips. Mesenchymal cells then aggregate at the tip of the UB to form an epithelial tubule that proceeds through several morphologic stages, including the renal vesicle,

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the comma and S-shaped bodies, and finally the nephron, an excretory unit of the kidney.^{1,6} The derivatives of the UB include collecting tubules, calyces, pelvis, and the ureters; the derivatives of MM include Bowman's capsule, glomeruli, proximal and distal convoluted tubules, and the loop of Henle.¹ The genes and signaling pathways involved in generating the overall architecture of the kidney include: *Lim-1*, *PAX2*, *OP-1*, *WT-1*, *WT-4*, *Sall1*, *Six1*, *Six2*, *GDNF*, *c-Ret*, *Wnt4*, *Osr1* and *Osr2*, *NPHS1* (neph- rin), *BMP-4*, *FGF*, *Notch*, and *JNK*.^{6–8}

The *Drosophila* malpighian tubules provide an excellent model tissue for studying cell fate specification, epithelial development, and physiology of renal systems and serves as an excellent model for studying human kidney diseases. The adult malpighian tubules consist of two pairs of epithelial tubes that empty into the hindgut at its junction with the posterior midgut. During embryogenesis, malpighian tubules develop from two sources: the ectodermal epithelial bud (hindgut primordium) and the mesenchymal mesoderm.^{2,9–11}

In the early stages of malpighian tubule development, interaction between the midgut and hindgut Anlagen redefines the expression of the transcription factor, Krüppel (*Kr*), to a small number of precursor cells. *Kr* further controls the expression of another transcription factor, *Cut*. The combined expression of the genes *Kr* and *Cut* provides for the evagination of malpighian tubules from the hindgut. Cell-signaling pathways also play an important role in the early and later developmental steps forming the malpighian tubules. These signaling pathways include wingless (*Wg*, cell allocation), hedgehog (*Hh*, bud evagination), TGF beta (*TGF-β*, branching of the tubules), *Notch* (*N*, defining the single tip cell), and *EGF* (*EGF*, elongation and cell division). Signaling from the tip cell through *EGF* is required for the proliferation of the distal cells of the tubule. The tubules then enter the vicinity of the caudal mesoderm to induce the mesoderm cells to undergo mesenchymal-epithelial transitions. The tubules derived from the initial ectodermal epithelium

consist of principal cells. Mesenchymal cells from the visceral mesoderm migrate and polarize along the epithelial tubules and incorporate into tubules as stellate cells.⁹

The malpighian tubules remain intact during metamorphosis but undergo some structural changes. The adult *Drosophila* has four malpighian tubules: a longer anterior pair and a shorter posterior pair, which converge through common ureters at the midgut-hindgut junction. Originally, only three regions (initial, transitional, and main segment) and two cell types (principal, or type I, cells and stellate, or type II, cells) were described in the malpighian tubules. However, genetic techniques for enhancer trapping, molecular marking, and clonal analysis revealed six regions and six cell types in the tubules.¹²

Each tubule is divided into four compartments: initial, transitional, main (secretory), and proximal (lower tubules and ureter also have reabsorptive properties). The initial, intermediate, and main segments of each tubule consist of two cell types comprising more than 150 cells. Type I cells in the malpighian tubules express *Cut* and is the major cell type (approximately 80%) found in the initial, transitional, main segment, and the region of lower tubules and ureters; type I cells transport cations and organic solutes. Type II cells expressing *Teashirt* (*TSH*), conduct water and chloride ions, and are found in the initial, transitional, and main segments, but not in the region of lower tubules and ureters. In addition, the proximal compartment (including the lower tubules and ureters) contains "tiny" cell types that collect the urine in the ureter and secrete neurohormones in the hemolymph to regulate ion transport.¹²

Despite major differences in general organization and physiology, mammalian kidneys and *Drosophila* malpighian tubules show clear similarities in development and function. Both use common strategies, such as the interaction of two distinct cell populations, one of which undergoes mesenchymal-epithelial transition. Even some of the pathways (*Wnt*, *TGF-β*, *FGF*, and *Notch*) and molecules (*Kr*- *Glis2*, *Cut*-*Cux-1*, *Brachyenteron-*

Brachyury, *Odd-Osr*, and *Hibris-Neph- rin*) are conserved in both systems through the development of tubules and their differentiation.⁹

STEM CELLS IN MAMMALIAN KIDNEY

Stem cells are fundamental to the self-renewal of adult tissues throughout the life of an organism. Adult stem cells found in many organs, including bone marrow, intestine, skin, and testes, which participate in the normal cell turnover of these organs, are a potential source of regenerative cells after tissue injury. The kidney, on the other hand, has a much lower rate of cellular turnover but has a great capacity to regenerate and proliferate after an ischemic injury. In animal models, glomerular structures that are completely lost can be repaired after immunologic injury.^{13,14}

However, the source of proliferating cells that repopulate injured nephrons or regenerate the lost renal tissues remains unknown. The cells that repopulate the tubule after injury may derive either from within the renal tubule (intrarenal) or from outside of the renal tubule (extrarenal). Three possible sources of stem cells contributing to renal tissue repair after injury have been suggested (Figure 1A).^{13,15,16} First, several groups have found cells from a distant site, such as bone marrow-derived mesenchymal stem cells or hematopoietic stem cells, may enter in the kidney and participate in the renal repair process, protecting the kidney from further injury.¹⁷ However, other groups have found no evidence of bone marrow-derived stem cells in two mouse models of bone marrow transplantation: one involving ischemic renal injury in male-to-female transplant recipients and the other involving a green fluorescence protein (GFP)-transgenic bone marrow donation to wild-type recipients.¹⁸ Furthermore, even if adult bone marrow-derived stem cells have the capacity to differentiate into renal tubular cells, the number of these cells is very low and does not seem to have a major role in tubular regeneration.¹⁹ Second,

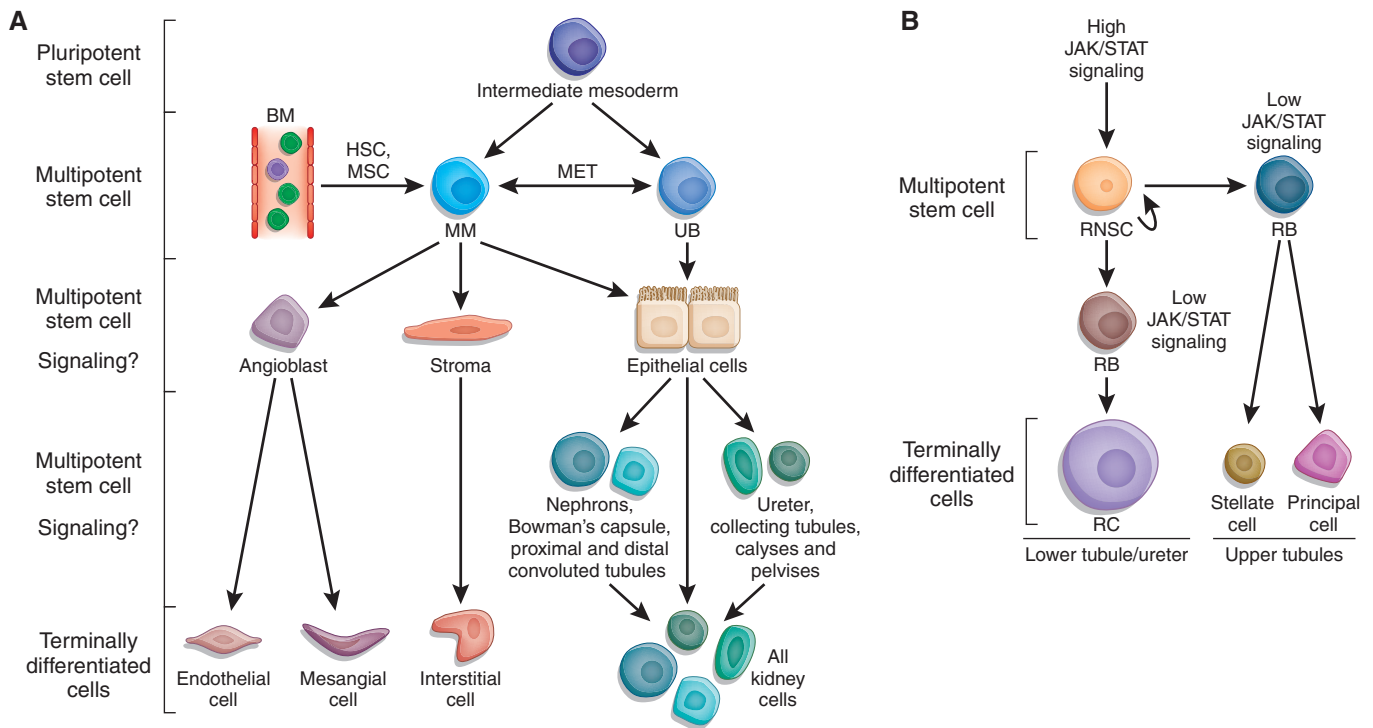


Figure 1. Proposed model of kidney stem cell lineages in mammals and *Drosophila*. (A) Schematic summary of lineage relationships in mammalian kidneys, where the metanephric mesoderm (MM) and ureteric bud (UB) share a common origin, the intermediate mesoderm. Kidney cells may also come from bone marrow-derived stem cells, or an epithelial cell precursor; whether these cells arise from a single stem cell or have a multiple independent lineage remains unclear. Further signaling and molecules regulating the stem cell lineage are also not clear. (B) Renal stem cell lineage in *Drosophila*. MET, mesenchymal-to-epithelial-transition; HSC, hematopoietic stem cell; MSC, mesenchymal stem cell; RNSC, renal and nephric stem cell; RB, renalblast; RC, renalcyte.

renal tubular injury may repair by the surviving tubular cells that lose their brush border and dedifferentiate into a mesenchymal phenotype, followed by migration, proliferation, and eventually redifferentiation into normal renal tubules.¹⁷ Lin *et al.*²⁰ genetically tagged renal epithelial cells with GFP and detected the co-expression of GFP with the mesenchymal protein vimentin in injured tissues, suggesting that surviving epithelial cells undergo dedifferentiation. Third, the renal papilla, a niche for adult kidney stem cells, contains many slow-cycling cells that are capable of migrating into the tubule and may differentiate into tubular epithelial cells in response to ischemic injury.²¹

Adult renal progenitor cells are also reported in other locations, such as the label-retaining cells among renal epithelia, multipotent progenitor cells from Bowman's capsule, the kidney side population along the proximal tubule, and CD133⁺ cells in the interstitium of adult human kid-

ney.^{19,22–24} Studies also suggest that two types of stem cell exist in renal tubules that give rise to all of the cell types in the adult kidney.²¹ A recent study in a rat model found a majority of cells in the proximal renal tubules are able to proliferate and contains the bulk of differentiated epithelial cells.²⁵ The identification of stem cells in other adult organs, together with evidence of kidney tissue repair or regeneration after injury, strongly suggests the presence of adult renal stem cells.

As differences in the findings of the above studies indicate, the exact source of renal stem cells and their location in the adult kidney are still not clear. At the same time, cell surface markers are also lacking or are less well characterized, making it difficult to isolate and purify stem cells from adult kidney. Recent studies suggest that the markers for adult kidney stem cells may include CD133⁺, PAX2⁺, CD45⁻, CD24a⁺, c-kit⁻, Ki-67⁺, and Sca-1⁺.²² Sca-1 and CD24a may reflect kidney side population

markers, and vimentin, CD90, PAX2, and Oct4 may identify multipotent renal progenitor cells.²² After ischemic injury, renal tubules undergo regeneration and repair, which is mediated by the paracrine release of growth factors from interstitial or endothelial cells, including EGF, HGF, TGF- α , and IGF-1.²⁶ Additionally, bone morphologic protein-7 (BMP-7) is able to repair severely damaged tubular epithelial cells and reverses chronic kidney injury.²⁷ However, despite this earlier work, little experimental evidence exists for determining the differentiation potential of renal stem cells, or identifying the signaling pathways or molecules that regulate adult kidney stem cell self-renewal or differentiation.

STEM CELLS IN MALPIGHIAN TUBULES

Most mammalian adult epithelial tissues, such as intestine, skin, cornea, and

mammary gland self-renew throughout adult life because of the presence of multipotent stem cells.²⁸ Studies have also confirmed the existence of adult stem cells in the reproductive system of *Drosophila*.^{29,30} However, the existence of multipotent cells in adult epithelial tissues from *Drosophila* did not become apparent until recently.^{31–33} Two new studies found the existence of a large number of multipotent stem cells in the adult *Drosophila* midgut^{31,32} that lie in close proximity to the extracellular matrix and to their immediate progeny. Further study found that Notch signaling regulates self-renewal or differentiation of these stem cells.^{31,32}

Like the regional interactions during development of mammalian kidneys, the malpighian tubules are derived from an interaction between hindgut primordial and visceral mesoderm through the process of mesenchymal-epithelial transitions and appears to be highly stable. The development of malpighian tubules in *Drosophila* is completed during embryogenesis. During metamorphosis, the larval gut degenerates, the gut entirely remodels, and the adult gut reforms. Notably, malpighian tubules do not remodel during metamorphosis and remain almost unmodified in the adult fly. We recently determined that the “tiny cells” in the region of the lower tubules and ureters of the malpighian tubules in adult *Drosophila* function as multipotent stem cells, capable of differentiating into all cell types in the malpighian tubules.³³

To identify stem cells in adult malpighian tubules, we reanalyzed the malpighian tubules and their cell types using known molecular markers. We performed lineage-tracing experiments incorporating BrdU (a proliferative marker), phosphorylated histone-3 (PH3; also a mitotic marker), and searched for new molecular markers to follow cell fate in the adult malpighian tubule over time. The details of how we characterized and differentiated the stem cells of the adult malpighian tubules, as well as how we determined the signaling required to control self-renewal or differentiation are discussed below.

First, we reanalyzed the cell types in

the malpighian tubules and found the principal cells have large nuclei and are distributed throughout the tubules, whereas the stellate cells have small nuclei and are restricted to the upper tubules, which include the initial, transitional, and main segments. Furthermore, we found that lower tubules and ureters are composed of three types of cells based on their nuclear sizes. The first type has a small nucleus and lies close to the cells of the tubular walls; these are similar to the previously reported “tiny” cells in the lower tubules and ureters.¹² The second cell type has a large oval nucleus and lies distant from the tubular walls. The third type has an intermediate-size nucleus and may be a transitional cell. We also found that cells of the malpighian tubules are different from those of the midgut because of difference in the expression of the markers in both systems; Cut and Kr express only in malpighian tubules, whereas Su(H) and Prospero express only in midgut.

To explore this work further, we have characterized the proliferating and mitotically active cells for additional molecular markers in the malpighian tubules. By incorporating BrdU (a proliferating marker) and staining for PH3 (a mitotic marker), we found that BrdU labels all three cell types in the lower tubules and ureters but not in the upper tubules. However, we only detected PH3 staining in cells with small nuclei, suggesting these cells with small nuclei are dividing and the cells with intermediate and large nuclei are undergoing endoreplication. Additionally, we characterized several other cell-specific molecular markers, such as *escargot* (*esg*), *kr* (transcription factor), and Armadillo (Arm; the β -catenin homolog), and found them expressed in small-nuclear-size cells in the lower tubules and ureters.

Next, we used a positively marked mosaic lineage labeling technique³⁴ to label and trace cells that undergo mitotic divisions. In this process, the production of GFP-marked clones relies on mitotic recombination, and we were able to see the proliferating cells directly. We observed the fate of the GFP-marked clones in malpighian tubules 2 to 10 d following

clonal induction and found that most basal diploid cells function as stem cells, which we term renal and nephric stem cells (RNSCs). The RNSCs were in contact with their immature diploid daughters, which we term the renalblasts. These renalblasts progress toward one of two fates. Some of the renalblasts become mature renalcytes in about 5 d through endoreplication in the lower tubules and ureters; in about 10 d, other renalblasts move toward the distal upper tubules to finally differentiate into principal and stellate cells in the transitional and initial segments. Using different molecular markers and clonal analysis, we also counted the number of RNSCs and found that, of 497 cells in each MT, one pair of anterior malpighian tubules has approximately 97 RNSCs.

Finally, we examined the expression pattern of the components of the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signal transduction pathway in malpighian tubules. We looked for signaling that regulates RNSC self-renewal or differentiation because such signaling regulates stem cell self-renewal in several other stem cell systems.^{35,36} The *Drosophila* JAK/STAT pathway has five main components: JAK, encoded by the *hopscotch* (*hop*) gene; STAT, encoded by the *stat92E* gene; a ligand encoded by the *unpaired* (*upd*) gene; a receptor encoded by the *domeless* (*dome*)/Master of Marrelle (Mom) gene; and a negative regulator, Socs36E.³⁷

The activated Stat92E enters the nucleus to activate the transcription of its target genes.³⁸ Using the positively marked mosaic lineage technique, we overexpressed the JAK/STAT ligand, Upd, and observed that overexpression of its signaling increases the size of the malpighian tubule and accelerates cell division of the RNSCs. Furthermore, using mosaic analysis with the repressible cell marker technique to analyze the STAT92E homozygous clones, we found absence of JAK/STAT signaling causes the loss of stem cell populations as well as the differentiation of RNSCs. Using a cell death marker, we further confirmed that differentiation of RNSCs is not the result

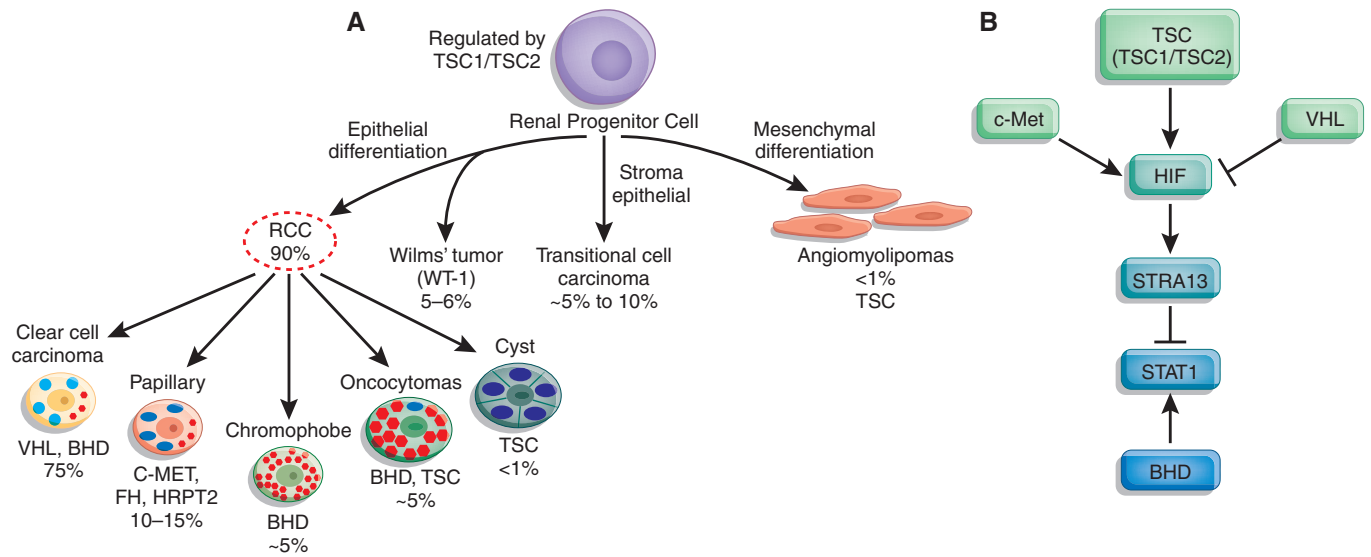


Figure 2. Schematic summary of genes/signaling regulating renal cell carcinomas (RCC) in mammalian kidney. (A) Model showing renal tumors associated with different genes. Renal progenitor cells are regulated by *TSCs* (*TSC1* and *TSC2*) genes that are able to differentiate into either mesenchymal or epithelial cells. Mutations in *TSC1* or *TSC2* cause either mesenchymal (oncocytomas and cyst) or epithelial (angiomyolipomas) tumors. However, most of the common tumors are epithelial carcinomas type. These tumors are clear cell carcinoma; papillary, chromophobe, oncocytomas; and some of the tumors of epithelial origin, only seen in children (Wilms' tumor); some of the tumors are also caused by the combination of both epithelial and stroma cells (transitional cell carcinoma). (B) Signaling pathways that regulate kidney tumors. TSC, tuberous sclerosis complex; TSC1, hamartin; TSC2, tuberin; VHL, von Hippel-Lindau; c-MET, met proto-oncogene papillary; FH, fumarate hydratase; BHD, Birt-Hogg-Dubé; HRPT2, hyperparathyroidism-2.

of cell death. Next, we checked the expression pattern of the JAK/STAT ligand, Upd, and its receptor (Dome) in RNSCs. Unlike other well-characterized adult stem cells in *Drosophila*, such as testis and ovary, where a fixed niche regulates stem cell self-renewal, in the malpighian tubule we found that both Upd and Dome express in RNSCs along with STAT92E protein. Based on this finding, we propose that self-renewal of RNSCs is controlled by autocrine JAK/STAT signaling. Thus, the RNSCs control their own self-renewal or differentiation in malpighian tubules and do not need a fixed niche. However, the RNSCs reside primarily in the lower tubules and ureters even in the Upd-overexpressing flies, suggesting, in addition to JAK/STAT signaling, that other signaling confines the RNSCs to the lower tubules and ureters. Furthermore, strong JAK/STAT signaling regulates RNSC self-renewal and weak JAK/STAT signaling prepares renalblasts for differentiation into renalcytes in the lower tubules and ureters and a principal or stellate cell in the upper tubules (Figure 1B). These findings in

the malpighian tubules of adult *Drosophila* suggest, perhaps, that only one type of stem cell is enough to repair and regenerate the damaged tissues in the mammalian kidney.

STEM CELLS IN RENAL CANCER

Normal development of the mammalian kidney requires controlled proliferation, cell-cell interactions, apoptosis, and differentiation, all in proper sequence and balance. A loss of this balance may facilitate some kidney diseases, including cancer.³⁹ Malignant tumors in many cases develop through the accumulation of clonal genetic changes in proliferating cells following activation of oncogenes, dysfunction of stability genes, or inactivation of tumor-suppressor genes. There are several varieties of kidney cancer, each with a different histology, producing different clinical courses, and each is associated with alterations in different genes.

Renal cell carcinoma is the most common type of kidney cancer, accounting

for more than 90% of malignant kidney tumors. Renal cell carcinoma originates primarily in proximal renal tubules and rarely in distal tubules. The five human genes associated with predisposition to renal cell carcinoma are von Hippel-Lindau (*VHL*; clear cell), met proto-oncogene (*c-MET*; papillary), fumarate hydratase (*FH*; papillary), Birt-Hogg-Dubé (*BHD*; chromophobe, oncocytomas, and clear cell), and hyperparathyroidism 2 (*HRPT2*; papillary) (Figure 2A). Renal carcinomas can also develop after chronic renal regeneration and repair in individuals with polycystic kidney disease or in renal allografts.

A detailed investigation³⁹⁻⁴¹ of kidney neoplasms suggests that some renal carcinomas, such as Wilms' tumor and hereditary papillary renal carcinoma, are caused by mutations in genes involved in normal nephrogenesis, such as WT-1 and c-met, respectively. Bilateral renal carcinoma is associated with the *TSC* gene and clear cell renal carcinoma with the *VHL* gene. With the exception of *TSC* mutations,⁴² all of the above-mentioned cancers are renal epithelial tumors,³⁹⁻⁴¹ which result from abnor-

malities of both mesenchymal cells (angio-myolipomas) and epithelial cells (cysts, oncocytomas, and carcinomas).

Many cancers are also considered stem cell diseases by some investigators because of their propagation by a minority of cells with stem-cell-like properties and their possible derivation from normal-tissue stem cells.⁴³ Furthermore, overlapping sets of molecules and pathways regulate both stem cell migration and cancer metastasis. Hypothetically, most adult stem cells reside in a quiescent state. However, the effect of persistent injury during chronic kidney disease might, over time, increase the pool size of stem cells in an active state of renewal and increase cancer incidence.⁴⁴ The greater the number of stem cells present in a tissue enhances the likelihood of an oncogenic event, particularly when a stem cell is trapped in an activated state by oncogenes.

To understand how cancer stem cells function in tumors, first we need to understand what signaling events regulates the formation of cancer cells. It has been proposed that TSCs (TSC1/TSC2) proteins regulate early renal progenitor cells.⁴² Individuals with TSC mutations have an increased incidence of renal cell carcinoma. TSC mutations activate the mammalian target rapamycin (mTOR) and biochemically resemble VHL alterations. The MET proto-oncogene, expressed in both stem and cancer cells, is a key regulator of invasive growth in normal conditions. VHL promotes the degradation of hypoxia-inducible growth factor-1 (HIF-1). HIF-1 induces the dedifferentiation of cancer cells, maintains stem cell identity, and increases metastatic potential.⁴⁵ VHL interacts with HIF-1 and the loss of VHL and overproduction of the HIF-1 contribute to the development RCC. Furthermore, genetic inactivation of VHL prevents HIF-1 down-regulation, leading to the expression of the MET proto-oncogene, an important regulator of invasion and metastasis. Moreover, VHL and HIF-1 pathways regulate STRA13, a cancer-associated protein overexpressed by many common malignancies. Both VHL deficiency and HIF-1 activation result in the repression of endogenous STAT1, which

possesses tumor-suppressor properties and is mediated by STRA13.⁴⁶ BHD is another dominantly inherited hamartoma syndrome⁴⁷ that shares several features with TSC; mutation in both genes causes RCC, which suggests that the BHD and TSC proteins may function within a common pathway.

Recently, we demonstrated in *Drosophila* that amplifying JAK/STAT signaling by overexpressing its ligand, Upd, stimulates the RNSCs to proliferate and differentiate into renalcytes, which results in oncogenic overgrowth of the malpighian tubule.³³ Previously, we had reported that BHD functions downstream of JAK/STAT signaling pathways and interacts with JAK/STAT to regulate germline stem cells in the *Drosophila* testis.⁴⁸ The genetic interactions of different tumor-suppressor genes regulating renal cell carcinoma are summarized in Figure 2B. We are exploring the possibility that the *Drosophila* RNSC system in malpighian tubules may be a handy *in vivo* system to study cancer stem cell regulation in nephrons. There remains the possibility that our system may also be useful in finding ways to restore normal clones by stem cell technology (cell-targeted therapy) and knowledge of developmental programming.^{21,49,50}

CONCLUSION

Despite several differences in general organizations and physiology, the *Drosophila* and mammalian kidney use strikingly parallel strategies in development and function. Genetic pathways and molecules that regulate kidney development are conserved in both systems. Development of normal adult kidneys requires controlled proliferation, cell-cell interactions, apoptosis, and differentiation in the proper sequence. Loss of this balance may lead the subsequent formation of kidney cancer, and understanding primitive developmental systems may be a window to explaining the clonal expansion of oncogenesis.

In *Drosophila* malpighian tubules, we have identified a multipotent stem cell that is able to generate all cell types of the adult. Furthermore, we found that auto-

crine JAK/STAT signaling regulates stem cell self-renewal or differentiation. However, important questions remain to be addressed, including whether there is a common pool of stem cells that generate a differential pattern of renal and midgut stem cells. To test this hypothesis, further experiments are needed to transfer renal stem cells to the midgut, and *vice versa*.

In the mammalian kidney, studies to determine the sources of nephron regeneration after injury and the existence of stem cells and their location have produced conflicting results. Additional clever experiments are needed to identify replacement cells during kidney regeneration. Understanding the function of multipotent kidney stem cells will undoubtedly lead to a better understanding of kidney development and has important clinical implications for early detection, prevention, and treatment of kidney diseases.

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DISCLOSURES

None.

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