

## Review

# Multipotent stem cells in the Malpighian tubules of adult *Drosophila melanogaster*

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### Summary

Excretion is an essential process of an organism's removal of the waste products of metabolism to maintain a constant chemical composition of the body fluids despite changes in the external environment. Excretion is performed by the kidneys in vertebrates and by Malpighian tubules (MTs) in *Drosophila*. The kidney serves as an excellent model organ to investigate the cellular and molecular mechanisms underlying organogenesis. Mammals and *Drosophila* share common principles of renal development. Tissue homeostasis, which is accomplished through self-renewal or differentiation of stem cells, is critical for the maintenance of adult tissues throughout the lifetime of an animal. Growing evidence suggests that stem cell self-renewal and differentiation is controlled by both intrinsic and extrinsic factors. Deregulation of stem cell behavior results in cancer formation, tissue degeneration, and premature aging. The mammalian kidney has a low rate of cellular turnover but has a great capacity for tissue regeneration following an ischemic injury. However, there is an ongoing controversy about the source of regenerating cells in the adult kidney that repopulate injured renal tissues. Recently, we identified multipotent stem cells in the MTs of adult *Drosophila* and found that these stem cells are able to proliferate and differentiate in several types of cells in MTs. Furthermore, we demonstrated that an autocrine JAK-STAT (Janus kinase–signal transducers and activators of transcription) signaling regulates stem cell self-renewal or differentiation of renal stem cells. The *Drosophila* MTs provide an excellent *in vivo* system for studying the renal stem cells at cellular and molecular levels. Understanding the molecular mechanisms governing stem cell self-renewal or differentiation *in vivo* is not only crucial to using stem cells for future regenerative medicine and gene therapy, but it also will increase our understanding of the mechanisms underlying cancer formation, aging and degenerative diseases. Identifying and understanding the cellular processes underlying the development and repair of the mammalian kidney may enable more effective, targeted therapies for acute and chronic kidney diseases in humans.

Key words: kidney, Malpighian tubules, kidney development, multipotent stem cells, renal and nephric stem cells, renal cancer, *Drosophila melanogaster*.

### Introduction

Excretion is an essential process in the life of an organism, involving the removal of the waste products of metabolism to maintain a constant chemical composition of the fluids even when the external environment changes. Unicellular organisms discharge their waste products directly through the cell surface, but multicellular organisms utilize more complex and specialized excretory organs. These organs vary in different animals but show a striking degree of similarity in form and function. For instance, excretion is performed by a single excretory cell in *Caenorhabditis elegans*, by Malpighian tubules (MTs) in insects, nephridia in annelids, rectal glands in sharks and kidneys in all other vertebrates. In mammals, the kidney is an important excretory and homeostatic organ, embedded in a connective tissue matrix of the body. It performs several essential biological functions to maintain the homeostatic balance of the bodily fluids by excreting and reabsorbing water and inorganic electrolytes; it also regulates blood pressure, glucose metabolism, erythropoiesis, synthesis of vitamin D and hormone secretion (Vainio and Lin, 2002; Dressler, 2006; Tomas and Kumar, 2008).

The MTs of *Drosophila melanogaster* together with the hindgut, acts as an excretory and osmoregulatory organ system. The MTs of the fly are free-floating within the hemocoelic body cavity (Dow et al., 1998). MTs perform several functions, including excreting

metabolic waste, excess water and organic metabolites (certain synthetic dyes); selectively reabsorbing the fluid in the proximal tubules of MTs and hindgut; and acting as an autonomous immune system (Maddrell et al., 1974; Denholm et al., 2003; Wang et al., 2004; Dow and Davies, 2006; Jung et al., 2005; Ruiz-Sanchez and O'Donnell, 2007; Dow, 2007a; Dow, 2007b; Evans et al., 2008; Day et al., 2008). Mammalian and fly kidney requires the proper integration and coordination in the development of specialized cell types within a well-defined architectural framework that functions in an integrated manner to maintain homeostasis of body fluids, electrolytes and nutrients (Jung et al., 2005).

### Common elements of renal development in mammals and *Drosophila*

The development of an organism requires proper integration and coordination of cell behavior and cell–cell communication. Reciprocal inductive interactions among cells result in cellular differentiation and formation of tissues and organs. The kidney serves as an excellent model organ to investigate the cellular and molecular mechanisms underlying organogenesis. It is an important organ system in which to analyze most of the basic developmental biology events, including epithelial–mesenchymal interaction, branching morphogenesis, cell polarization and pattern formation and differentiation (Vainio and Lin, 2002; Jung et al., 2005; Dressler, 2006).

The mammalian kidney develops from the intermediate mesoderm and proceeds through three successive stages of development, each marked by the development of a more advanced pair of organs: the pronephros, the mesonephros and the metanephros. The adult metanephros develops by cycles of ureteric bud (UB) branching and nephron formation. The cycles begin and are maintained by reciprocal inductive interactions and by feedback between epithelial UB tips and the surrounding metanephric mesenchyme (MM). In the process of these interactions, branching of the UB is dependent on the MM, and the differentiation of the nephrons is integrated with each new collecting tubule (Saxen, 1987; Vainio and Lin, 2002; Dressler, 2006). The epithelial UB emanates from the Wolffian duct (WD), grows toward and invades the adjacent MM, and then undergoes multiple interactions of a branching program [mesenchymal-to-epithelial transition (MET)] that leads to the development of an extensive urinary collecting system (Dressler, 2006; Rosenblum, 2008). MM cells then condense around the tips of the growing and branching ureter and aggregate at the tip of the UB to form an epithelial tubule that proceeds through several morphological stages and ultimately gives rise to the nephron, an excretory unit of the kidney (Fig. 1) (Saxen, 1987; Vainio and Lin, 2002; Wang et al., 2004; Jung et al., 2005; Dressler, 2006; Rosenblum, 2008). The Bowman's capsule, glomeruli, proximal and distal tubules arise from the MM and the collecting ducts and the ureter arises from the UB (Fig. 1). The continuous MET generates ~12,000 nephrons in the adult mouse kidney and ~500,000 to 1,000,000 nephrons in the adult human kidney. Molecular genetic analyses of the mammalian system have revealed a complex network of more than 400 genes that control kidney organogenesis. These include the transcription factors, signaling pathways, growth factors molecules, and transmembrane protein and extracellular matrix molecules (Dressler, 2006; Park et al., 2007; Yokoo et al., 2008; Uhlenhaut and Treier, 2008). Elucidating the cells and the molecular basis of signaling events involved during development will increase our understanding of the molecular basis of hereditary and sporadic renal developmental defects, and lead to useful new diagnostic and therapeutic tools that can be used to treat kidney diseases and manipulate kidney function in adults.

The *Drosophila* MTs provide an excellent model organ for studying cell fate specification, organization of epithelial sheets and physiology of renal systems, and serves as an excellent model for

studying human kidney diseases (Dow and Davies, 2006; Chintapalli et al., 2007). MTs develop from two sources: the ectodermal epithelial bud (hindgut primordium) and the mesenchymal mesoderm. The MTs consist of monolayer epithelial cells with a distinct apical-basal polarity. The development of *Drosophila* MTs, which is largely completed during embryogenesis, includes an allocation of tubule primordia and then tube budding, followed by growth and elongation *via* cell rearrangements and, eventually, cell differentiation (Ainsworth et al., 2000; Denholm et al., 2003; Jung et al., 2005; Dow, 2007a; Dow, 2007b). In the early stages of MT development, interaction between the midgut and hindgut anlagen redefines the expression of the two transcription factors, zinc-finger transcription factor, Krüppel (Kr) and homeodomain-containing protein, Cut in primordial cells (Gaul and Weigel, 1990; Liu and Jack, 1992). Their genes are expressed in the tubule primordia independently and they act together to specify tubule cell fate (see Hatton-Ellis et al., 2007). Following tubule evagination, extension of the cylindrical buds into crescent-shaped tubes requires *ribbon* (*rib*), which encodes a BTB/POZ-type nuclear protein, *faint sausage* (*fas*), which encodes an extracellular, immunoglobulin-like protein, and *ret* (Hahn and Bishop, 2001). The tubule cell-shape changes, and the rearrangement of the cytoskeleton leads to tube elongation, which is regulated by the *walrus*, *zipper*, *hibris*, *rib*, *rolling pebbles*, *myoblast city* and *rac* genes (Liu et al., 1999). Signaling from the tip cell is required for the proliferation of the distal cells of the tubule. The four MTs are derived from two cell populations: ectodermal epithelial buds and the surrounding mesenchymal mesoderm (Sozen et al., 1997). The tubules derived from the initial ectodermal epithelium buds consist of principal cells (PC). Mesenchymal cells from the visceral mesoderm migrate and polarize along the epithelial tubules, where they undergo a MET and incorporate into tubules as stellate cells (SC) that require *hibris*, an ortholog of the vertebrate Nephhrin (Denholm et al., 2003). Several genes and signaling pathways are known to play a crucial role in the early and later developmental events underlying the formation of functional MTs (Sozen et al., 1997; Wan et al., 2000; Ainsworth et al., 2000; Sudarsan et al., 2002; Denholm et al., 2003; Dow and Davies, 2006; Hatton-Ellis et al., 2007; Evans et al., 2008).

Mammalian kidneys and *Drosophila* MTs show distinct similarities in development and function. The development of both systems involves the interaction of two distinct cell populations,

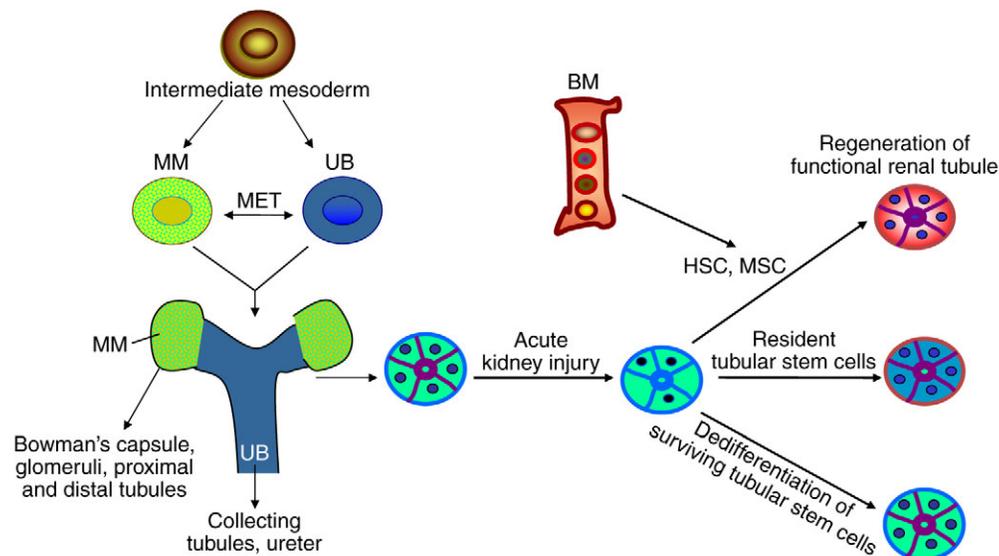


Fig. 1. Proposed model of mammalian kidney development and the origin of tubular epithelial regenerating cells after acute renal injury. MM, metanephric mesenchyme; UB, ureteric bud; MET, mesenchymal-to-epithelial transition; BM, bone marrow; HSC, hematopoietic stem cells; MSC, mesenchymal stem cells.

one of which undergoes MET to acquire key functional units. Even some of the pathways and molecules are conserved in both systems during development (Ainsworth et al., 2000; Denholm et al., 2003; Jung et al., 2005). The knowledge gained from the development and physiological function of *Drosophila* MTs will provide a unique insight into the role of the cellular pathways required in mammalian renal development and eventually lead to a better understanding of complex renal diseases.

#### Stem cells in regeneration and repair of mammalian kidney

In recent years, stem cells have emerged as one of the fundamental backbones of tissue biology. Stem cells are defined functionally as unspecialized cells that have an unlimited capacity to self-renew through mitotic cell division, as well as the ability to differentiate into a diverse range of specialized cell types that support normal development and tissue homeostasis. Both the self-renewal and differentiation processes must be tightly regulated by two types of proliferative behavior, asymmetric and symmetric division, to ensure the survival of an organism (Yamanaka, 2007; Morrison and Spradling, 2008; Yamashita and Fuller, 2008). These unique properties of stem cells will provide a powerful tool for future regenerative medicine and gene therapy. Growing evidence suggests that stem cell self-renewal and differentiation is controlled by both intrinsic and extrinsic factors, and that misregulation of stem cell behavior results in cancer formation, tissue degeneration and premature aging.

In mammals, two kinds of stem cells, embryonic and adult, have been identified, and each has distinct functions and characteristics. Embryonic stem cells are considered as true stem cells, as they generate mature progeny of all cell types; by contrast, the adult stem cells are thought to have limited proliferation potential and can only differentiate into the mature tissue in which they reside. However, the accumulative evidence suggests that adult stem cells may have greater plasticity than previously recognized. Despite their extensive proliferative capacities, stem cells may be quiescent *in vivo* until injury or tissue degradation stimulates the regenerative signal. The adult stem cells may repair the damaged tissues by differentiating into appropriate cell phenotypes, by providing cytokines and other factors to enhance recovery of endogenous cells, or by undergoing cell fusion (Morris and Spradling, 2008). Adult stem cells play an important role in cell turnover and regeneration. However, several obstacles exist in the use of adult stem cells as disease therapy. First, the ability to identify most adult stem cells is hampered by the lack of stem cell markers. Second, *in vitro* systems for manipulating adult stem cell populations are often not well defined. Finally, our understanding of how adult stem cells are regulated within their niche is still far from complete. Multipotent adult stem cells have been reported in many organs, such as bone marrow, skin, intestine, prostate, pancreas, lung and testes, with unrestricted potential to form different cell types after tissue injury (Blanpain et al., 2007; Barker et al., 2008).

Adult stem cells play an important role in cell turnover and regeneration. The kidney, however, has a low rate of cellular turnover but has a great potential for tissue regeneration following an ischemic or toxic injury. Ischemic injury to the kidney causes acute renal failure, loss of tubular polarity, necrosis and cell death, followed by tubular regeneration and recovery of renal function (Anglani et al., 2008; Gupta and Rosenberg, 2008; Yokoo et al., 2008; Vaidya et al., 2008). In humans, two broad categories of kidney disease have been characterized: acute and chronic. Acute renal failure is a leading cause of morbidity and mortality. Developmental studies reveal that the kidney has more than 26

terminally differentiated cell types, suggesting that differentiation potential would be helpful in renal regeneration after injury. Many animal models provide evidence of regenerating completely degenerated renal tissues after injury (Elger et al., 2003; Haller et al., 2005), which can help us understand the cellular and molecular mechanisms of ischemic injury and tubular regeneration (Imai et al., 2007; Gupta and Rosenberg, 2008; Yokoo et al., 2008; Vaidya et al., 2008). There is an ongoing debate about the source of proliferating cells (stem cells) in the adult kidney that repopulate injured nephrons or regenerate the lost renal tissues. Whether the adult stem cells that repopulate the renal tubule following injury come from within the renal tubule or from extra-renal cells remain controversial. Kidney regeneration and repair occur through three possible sources of stem cells: dedifferentiation of surviving tubule cells, bone marrow-derived stem cells or resident kidney stem cells (Fig. 1) (Oliver et al., 2004; Cantley, 2005; Duffield et al., 2005; Gupta et al., 2006; Imai et al., 2007; Imberti et al., 2007; Bussolati et al., 2008; Gupta and Rosenberg, 2008; Yokoo et al., 2008; Vaidya et al., 2008). Stem cell-based renal regeneration would be critical in reducing the incidence and severity of acute renal failure and treatment of several other kidney diseases and cancer.

Several studies in preclinical models of acute and chronic kidney injury have demonstrated that cells from bone marrow (bone marrow-derived stem cells, mesenchymal stem cells) may migrate to the kidney and participate in the generation of new epithelial cells following injury (for details, see Gupta and Rosenberg, 2008; Sagrinati et al., 2008). Mesenchymal stem cells have also been found to produce several growth factors such as VEGF, HGF, IGF-1, BMP-7, and TGF- $\alpha$ , suggesting that, after renal injury, a paracrine effect of renal vasculature may provide regeneration and repair. Further studies have examined the role of bone marrow-derived stem cells in renal regeneration, with different results (Gupta and Rosenberg, 2008; Sagrinati et al., 2008). Moreover, several recent studies have shown that tubular cell replacement with bone marrow-derived stem cells occurs less frequently than previously thought, suggesting that these cells may not represent a major tool in cell therapy after tubular injury and that regenerative cells originate from intra-renal cells (Lin et al., 2005). A recent study even suggests that treatment of renal failure with bone marrow stem cells can be offset by a partial maldifferentiation of bone marrow stem cells into adipocytes, resulting in glomerular sclerosis (Kunter et al., 2007). Thus, kidney-specific stem cells may be better for tissue replacement because of their inherent organ-specific identity, which can reduce the risk of maldifferentiation. Several other studies suggest that renal tubular injury may be repaired by the less damaged cells, which can migrate, proliferate and ultimately repopulate into normal renal tubules by the dedifferentiation (Lin et al., 2005; Humpherys et al., 2008).

Accumulative evidence suggests that the repair of kidney injuries is predominately regulated by different endogenous renal stem cells (Table 1). However, the location and behavior of renal stem cells remain controversial. Adult kidney stem cells have been isolated using four different selection strategies that have been used to successfully isolate stem cells from other organs (Gupta and Rosenberg, 2008). (1) BrdU (5-bromo-2-deoxyuridine) labeling. Because stem cells are slow-cycling cells and they can retain the label for a long period of time and it has been shown that label-retaining cells function as a source of regenerating cells in adult kidney (Maeshima et al., 2003). These cells remain, for a long period of time, at the interstitial cells of renal papilla, and are multipotent *in vitro* (Oliver et al., 2004). However, a recent study used the genetic fate mapping technique in mice to exclude the presence of stem cells in the interstitial renal cells, and the results indicate that

Table 1. Isolation and localization of the multipotent stem cells in mammalian and *Drosophila* kidney

Species	Isolation method	Markers	Localization	Potential	References
Rat	Label retaining/marker	BrdU, HIF1 $\alpha$	Papilla	Multipotent	Oliver et al., 2004
Rat	Label retaining	BrdU	Proximal tubule	Unipotent	Maeshima et al., 2003
Rat	Side population	Sca1, Kit, CD45	Proximal tubule	None	Iwatani et al., 2004
Mouse	Side population	Sca1, muscudin/MyoR	Interstitial	Multipotent	Hishikawa et al., 2005
Mouse	Side population	Sca1, CD24, endoglin/CD105, Kit, Ki67	Proximal tubule	Multipotent	Challen et al., 2006
Mouse	Molecular marker	Sca1	Interstitial of papilla	Multipotent	Dekel et al., 2006
Human	Molecular marker	CD133, Pax-2, CD44	Interstitial	Pluripotent	Bussolati et al., 2005
Human	Molecular marker	CD133, Oct-4, CD24, BMI1	Bowman's capsule	Multipotent	Sagrinati et al., 2006
Mouse	Molecular marker	CD29, CD44, CD73, CD90	Glomeruli	Multipotent	da Silva Meirelles et al., 2006
Rat	Culture	Pax2, Sca1, musashi 1, Wnt1, Wnt4	S-3 segment of proximal tubule	Multipotent	Kitamura et al., 2005
Rat	Culture	Oct4, Rex1, Pax2, CD44	Proximal tubule	Multipotent	Gupta et al., 2006
Rat/human	Label retaining, molecular marker	BrdU, cyclin D1, Ki-67	Proximal tubule	None	Vogetseder et al., 2008
Mouse	Lineage labeling	BrdU, Six2, Ki67	Tubular epithelial cells	Multipotent	Humphreys et al., 2008
Mouse	Subcapsular implant, molecular marker	Sca-1, prominin-1, aquaporin-2	Papilla cortex, outer medulla	Multipotent	Curtis et al., 2008
Mouse/human	Molecular marker	(SDF1), CXCR4, CXCR7	Bowman's capsule	Multipotent	Mazzeinghi et al., 2008
<i>Drosophila</i>	Lineage labeling, molecular marker	BrdU, PH3, Armadillo, Unpaired, STAT92E, Escargot, Kruppel, TSH, Cut	Lower tubules and ureters	Multipotent	Singh et al., 2007

surviving tubule epithelial cells are the predominant mechanism of adult mammalian kidney repair after ischemic injury (Humphreys et al., 2008). (2) Isolation of side-population (SP) cells because these cells extrude Hoechst dye through the activity of multidrug resistance proteins of the ATP-binding cassette transporter superfamily (Gupta and Rosenberg, 2008). SP cells (Hoechst low cells) isolated from many different organs are multipotent stem cells (Challen and Little, 2006). Stem cells are also found in the SP cells of the adult kidney with multilineage differential potential (Iwatani et al., 2004; Challen et al., 2006). (3) The identification and isolation of kidney stem cells using specific cell surface markers that have been used to identify stem cells in other organs including kidney. Gupta et al. (Gupta et al., 2006) identified multipotent renal stem cells in the proximal tubules of the rat kidney and showed that these cells express several markers, including CD90, Oct4 and Pax2. Recently, multipotent renal stem cells have been identified in the adult human kidney in a subset of parietal epithelial cells located in Bowman's capsule (Bussolati et al., 2005; Sagrinati et al., 2006). These progenitor cells have the ability to self-renew and differentiate into several cell types in the kidney, and are characterized by the expression of stem cell markers, such as CD24, CD133, SDF1, CXCR4 and CXCR7 (Sagrinati et al., 2006; Mazzeinghi et al., 2008). These findings in the adult human kidney suggest that CD24, CD133, SDF1, CXCR4 and CXCR7 play an essential role in the therapeutic homing of human renal progenitor cells in acute renal failure, with important implications for the development of stem cell-based therapies for renal injury (Mazzeinghi et al., 2008). (4) Using specific culture conditions that have been used widely in selecting stem cells in other organ systems it has been shown that multipotent renal progenitor cells isolated from kidney express the stem cell markers and can differentiate into mature tubular cells (Kitamura et al., 2005; Gupta et al. 2006).

The characterization of stem cells in other adult organs, together with evidence from animal and human models for renal tubule repair or regeneration after injury, strongly suggests the presence of adult renal stem cells. However, the precise source and location of renal stem cells in the adult kidney remain unclear. Subsequently, the stem cell markers in the adult kidney are also lacking or less well characterized, making it difficult to isolate, to define a renal stem

cell niche, or to follow cell lineage progression in the normal or injured kidney (Gupta and Rosenberg, 2008). A summary of the isolation and localization of kidney stem cells, including candidate markers, in different species is presented in Table 1. However, precise identification and characterization of adult kidney stem cells and signaling pathways regulating their self-renewal or differentiation are poorly understood. Additionally, clonal analysis might be essential for comparing the different mechanisms underlying renal repairs. Understanding the signaling pathways regulating the proliferation and differentiation of adult kidney stem cells will undoubtedly provide the development of novel therapeutic strategies for the treatment of acute kidney injury (Humphreys et al., 2008).

#### Stem cells in the renal tubules of adult *Drosophila*

The development of *Drosophila* MTs is completed during embryogenesis. The MTs remain intact during metamorphosis but undergo some structural changes. Tissue homeostasis is critical for the maintenance of adult tissues. Most, if not all, adult epithelia of mammals and MTs have a well-defined organizational structure. Epithelial tissue can be classified into two broad categories based on turnover time: rapidly self-renewing and persistent. Most of the mammalian adult epithelial tissues, such as intestine, skin, cornea and mammary gland, undergo rapid self-renewal and replace damaged or dead cells throughout the life of the animal. Studies on epithelial tissue turnover suggest that cell self-renewal varies among different types of epithelial tissues. *Drosophila* is a highly attractive model system for the study of several important, complex biological processes, including epithelial tissue turnover.

Historically, the adult organs of *Drosophila* have been viewed as strictly postmitotic. Detailed studies exist on the adult stem cells of the reproductive system of *Drosophila* (see Hou and Singh, 2008). However, the existence of multipotent cells in adult *Drosophila* epithelial tissues did not become clear until recently (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006; Ohlstein and Spradling, 2007; Singh et al., 2007; Takashima et al., 2008). Recent studies have shown that the *Drosophila* midgut contains many multipotent stem cells that lie close to the extracellular matrix, and Notch signaling regulates self-renewal or differentiation of these

stem cells (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006; Ohlstein and Spradling, 2007). Additionally, there is an age-related increase in the number and activity of midgut stem cells and progenitor cells, regulated by PDGF and/or VEGF (Choi et al., 2008). Another study has identified adult stem cells in hindgut of *Drosophila* that are regulated by Wingless (Wnt), Hedgehog (Hh) signaling (Takashima et al., 2008). During metamorphosis, the larval gut degenerates, the gut is entirely remodeled, and the adult gut is formed. However, the Malpighian tubules are not remodeled during metamorphosis and remain almost unmodified in the adult. How cell turnover is managed in the adult MT cells and what factor(s) regulates MT cell self-renewal were unknown until we identified the cells with small nuclei in the region of the lower tubules and ureters of the MTs in adult *Drosophila*. These stem cells function as multipotent stem cells, capable of differentiating into all cell types in the tubule and regulated by an autocrine JAK-STAT signaling (Fig. 2A–H) (Singh et al., 2007; Singh and Hou, 2008). The details of the identification and characterization of the behavior of stem cells in the renal tubules of the adult *Drosophila* are described below.

#### Identification and localization of renal stem cells in Malpighian tubules

The adult MTs consist of two pairs of epithelial tubes: a longer, anterior pair that runs through the hemolymph on both side of the midgut, and a shorter, posterior pair that runs along the hindgut. These pairs converge in the ureter at the midgut–hindgut junction (Fig. 2A). Initially, only three regions (initial, transitional and main segments) and two cell types (principal, or type I, cells, and stellate, or type II, cells) were described in the MTs. However, genetic

techniques of enhancer trapping, molecular marking and clonal analysis revealed six regions and six cell types in the tubules (Sozen et al., 1997; Dow and Davies, 2006; Hatton-Ellis et al., 2007). These regions are highly specified, and the cells have a precise positional identity as a result of the interaction of the combinations of transcription factors (Wang et al., 2004). Each tubule is divided into four compartments (Fig. 2A): initial, transitional, main (secretion) and proximal (lower tubules and ureter reabsorption). The initial, intermediate and main segments of each tubule consist of two cell types consisting of more than 150 cells. Type I cells in the MTs express Cut, and make up the majority (~80%) of the cells found in the initial, transitional and main segments, and the region of lower tubules and ureters; Type II cells transport cations and organic solutes. Type II cells that express 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 (Wessing and Eichelberg, 1978; Sozen et al., 1997). In addition, the proximal compartment (including the lower tubules and ureters) contains ‘tiny’ cell types, homologs of myoendocrine cells in the ant *Formica*, that collect the urine in the ureter and secrete neurohormones in the hemolymph to regulate ion transport (Sozen et al., 1997). However, because a detailed analysis of the different types of cells in the proximal tubules had not been performed, we reanalyzed the different cell types in the MTs using techniques to identify known molecular markers that had been shown to be expressed in MTs, such as an antibody to Cut and a Tsh-*lacZ* enhancer trap line (Sozen et al., 1997). As reported previously, we found that principal cells, express Cut, have large nuclei and are distributed throughout the tubules, whereas the Tsh-positive cells are SC, have small nuclei

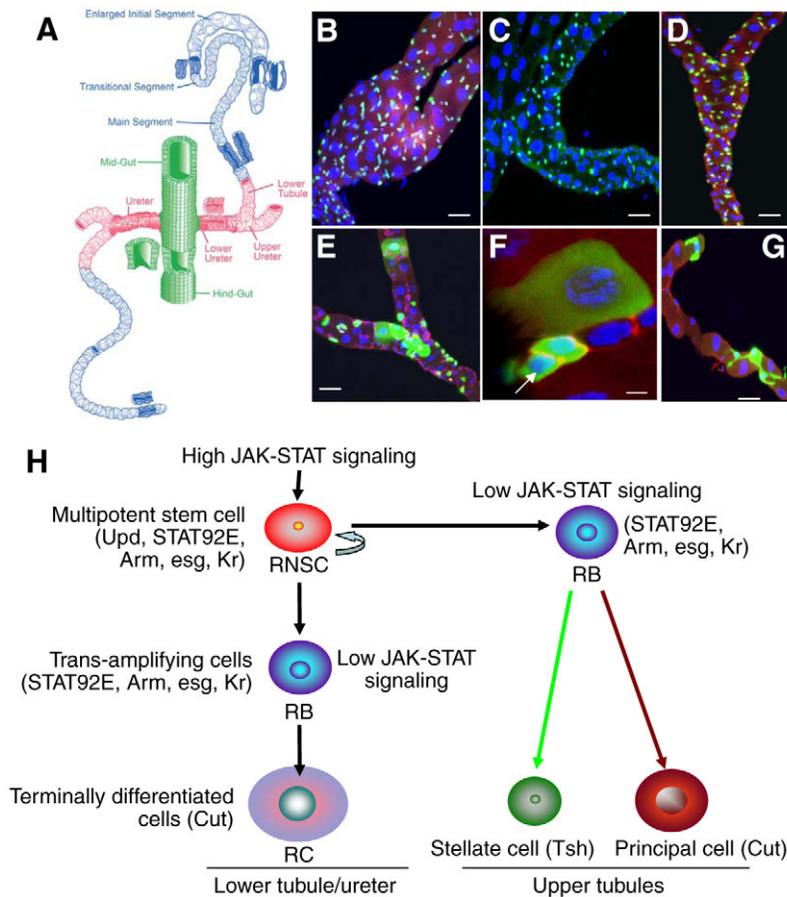


Fig. 2. The Malpighian tubules (MTs) and renal stem cell lineage in *Drosophila*. (A) Drawing of the *Drosophila* MTs (modified from Wessing and Eichelberg, 1978). The adult MTs consist of two pairs of epithelial tubes: a longer, anterior pair runs through the hemolymph on both sides of the midgut, and a shorter, posterior pair runs along the hindgut. The pairs converge at a common ureter at the midgut–hindgut junction. Each tubule is divided into four compartments: initial, transitional, main and proximal (lower tubules and ureter). (B–D) Expression pattern of unique molecular markers in the region of lower tubules and ureters of adult *Drosophila* MTs. (B) *Kr-Gal4/UAS-GFP* is specifically expressed in the small nuclear cells (anti-GFP, green; anti-Arm, red; DAPI, blue). (C) *upd-Gal4/UAS-GFP* expressed in the region of the lower tubules and ureters (anti-GFP, green; DAPI, blue). (D) *Stat92E-GFP* reporter is expressed only in small nuclear cells in the region of the lower tubules and ureters (anti-Arm, red; anti-GFP, green; DAPI, blue). (E–G) MTs with GFP-marked wild-type MARCM clones. (E) Six days after clone induction, GFP marks clusters of cells with small, intermediate and large nuclei in the region of the lower tubules and ureters. (F) An enlarged view of a GFP-marked clone from E [arrow, renal and nephric stem cells (RNSCs), anti-arm, red; anti-GFP, green; DAPI, blue]. (G) 10 days after clone induction in the upper tubule, the GFP labels both stellate and principal cells. (H) Schematic summary of renal and nephric stem cell lineage in *Drosophila* MTs (modified from Singh and Hou, 2008). Markers expressed in RNSCs, and their differentiated cells are shown in parentheses. RB, renblast; RC, renalcyte. Scale bars, 10  $\mu$ m (B–E), 5  $\mu$ m (F–G).

and are restricted to the upper tubules that include the initial, transitional and main segments. Furthermore, using phalloidin and DAPI staining in the tubule, we found that three types of cells, each with a different-sized nucleus, occupy distinct positions in the region of lower tubules and ureters. The first type has a small nucleus and lies primarily close to the tubular walls and is similar to the previously reported 'tiny' cells in the lower tubules and ureters; the second type has a large, oval nucleus and is distant from the tubular walls. The third type of cell has an intermediate-size nucleus and may be a transition-type cell. We also found that cells of the MTs are different from those of the midgut because of the difference in the expression of certain proteins in both systems [Cut and TSH are expressed only in the MT; Su(H) (Suppressor of Hairless) and Prospero are expressed only in the midgut].

We also asked whether cells in the MTs are proliferating and mitotically active throughout adult life. The epithelial cells can be labeled by continuous incorporation of the nucleotide analog BrdU for a long period of time; during the chase period, the label in the cells is diluted through cell division. The more slowly dividing cells, however, can retain the labeling for a longer period, and cell division can be observed in postmitotic tissues. We found that when BrdU (a proliferating marker) is incorporated, it labels all three cell types in the lower tubules and ureters. However, no BrdU-labeled cells have been seen in the upper tubules. Since many cells undergo endoreplication in both larvae and adult tissues, the BrdU can label both endoreplicating and dividing cells. To distinguish endoreplicating cells from dividing cells in the MTs, we stained the tissue for phosphorylated-histone-H3 (H3P), which plays an important role in gene expression, chromatin remodeling, chromosome condensation and cell division. H3P may initiate at different phases of the cell division in different organisms, but metaphase chromosomes are always found to be heavily phosphorylated. H3P is used as a mitotic marker in a wide range of organisms. In contrast to BrdU labeling, which occurs in all three cell types in the lower tubules and ureters, we detected H3P staining in cells with small nuclei, suggesting that the cells with small nuclei divide and the cells with intermediate and large nuclei undergo endoreplication. Additionally, we characterized several cell-specific molecular markers, such as two transcription factors, *Esg* (Escargot), and *Kr* and a membrane protein (Fig. 2B), *Arm* (Arm; the  $\beta$ -catenin homolog), which is expressed in small-nuclear-sized cells in the lower tubules and ureters. Furthermore, we found that both *Esg* and *Kr* sometimes present in a pair of H3P-positive dividing cells. Since the function of the *esg* gene is to maintain cells as diploid in *Drosophila* imaginal cells, the *Esg*-positive cells in the MTs are most probably diploid cells.

#### Cells with small nuclei in the proximal tubules of MTs are multipotent stem cells

To determine whether stem cells maintain cell turnover in the MTs, and if so, whether stem cells are able to recruit different types of cells to the tubule, we used a positively marked mosaic lineage (PMML) labeling technique (Kirilly et al., 2005) to label and trace cells that undergo mitotic divisions. Genetic lineage labeling in *Drosophila* involves using heat shock to induce a mitotic genetic recombination event that results in permanent expression of an easily visualized reporter transgenes [such as GFP (green fluorescent protein) or *lacZ* ( $\beta$ -galactosidase)] in a proliferating cell as well as in all progeny of that cell. Because the recombination event is infrequent, only single cells in a small area are labeled, and upon proliferation of these cells over time, the labeled cells make a small cluster. We heat-shocked the flies and observed the fate of the GFP-marked clones in adult MTs from 2–10 days after clone induction.

By 2 days after clone induction, GFP had labeled a few cells with small nuclei in the basal region of the ureter and lower tubules, and we observed a few transient clones of cells with large nuclei and limited proliferation potential. By 4–6 days after clone induction, clones were restricted mainly to the region of the lower tubules and ureter, with individual cells with small nuclei and clusters of cells with small, intermediate and large nuclei. We found that most basal diploid cells with small nuclei function as stem cells, and we term these the renal and nephric stem cells (RNSCs). The RNSCs contacted their immature diploid daughters, which we term the renalblasts (RBs), and these RBs underwent two fates: in the lower tubules and ureters, the RBs began to increase in size and DNA content, to become cells with intermediate and large nuclei in ~5 days, and express Cut. We term the intermediate and large nucleus cells early and late renalcytes (RCs), respectively (Fig. 2E–F,H). Ten days following clone induction, the RBs moved toward the distal upper tubules and finally differentiated into Cut-positive PCs and Tsh-positive SCs in the transitional and initial segments (Fig. 2G,H). Using different molecular markers and clonal analysis, we also found that, out of ~500 cells in one pair of anterior MTs, >90 cells were RNSCs. This analysis suggests that cells with small nuclei in the regions of the lower tubules and ureters function as multipotent stem cells in the renal tubules because they are able to proliferate and differentiate into several types of cells (Fig. 2E–H).

#### Regulation of stem cell behavior in MTs

Understanding what regulates stem cell behavior will allow us to predict behavior during development and homeostasis, and after disease. Additionally, determining how signaling pathways or molecules regulate stem cell self-renewal and differentiation is a critical step toward manipulating adult stem cells for therapeutic purposes. The various stem cell niches found in organisms from insects to mammals appear to have common signal transduction pathways, including the JAK-STAT, canonical Wnt, Hh and Notch pathways (Li and Xie, 2005; Ohlstein and Spradling, 2007; Hou and Singh, 2008; Morrison and Spradling, 2008; Takashima et al., 2008; Nusse, 2008). The existence of a stem cell niche has been proposed for several adult stem cell systems (Martinez-Agosto et al., 2007; Jones and Wagers, 2008). The precise spatial organization of the stem cells and the surrounding supporting cells is important to provide the proliferative signal and to avoid factors that enhance differentiation. Maintaining the balance between proliferation-inhibiting and proliferation-stimulating signals is the key to maintaining tissue homeostasis. Adhesion between stem cells and support cells plays an important role in keeping stem cells within the niche so that they may receive the self-renewal signal. Additionally, the niche may provide the polarity signal, which orients stem cells within the niche (Yamashita and Fuller, 2008). Stem cell niches are well characterized in the adult testis and ovary of *Drosophila*, where a fixed niche regulates stem cell self-renewal (Kiger et al., 2001; Lin, 2002; Brawley and Matunis, 2004; Li and Xie, 2005; Decotto and Spradling, 2005; Wang et al., 2006; Nystul and Spradling, 2007; Hou and Singh, 2008; Voog et al., 2008). However, in the MTs, the renal stem cells do not adhere to a particular cell type and are scattered over the region of the proximal tubules. If there is no fixed niche for renal stem cells in MTs, what factor(s) regulate their self-renewal and differentiation? To determine what signaling or molecules regulate renal stem cells behavior in MTs, we examined the expression pattern of the JAK-STAT pathway components because the signaling regulates stem cell self-renewal in several other stem cell systems. The *Drosophila* JAK-STAT pathway components are illustrated in Fig. 3. These

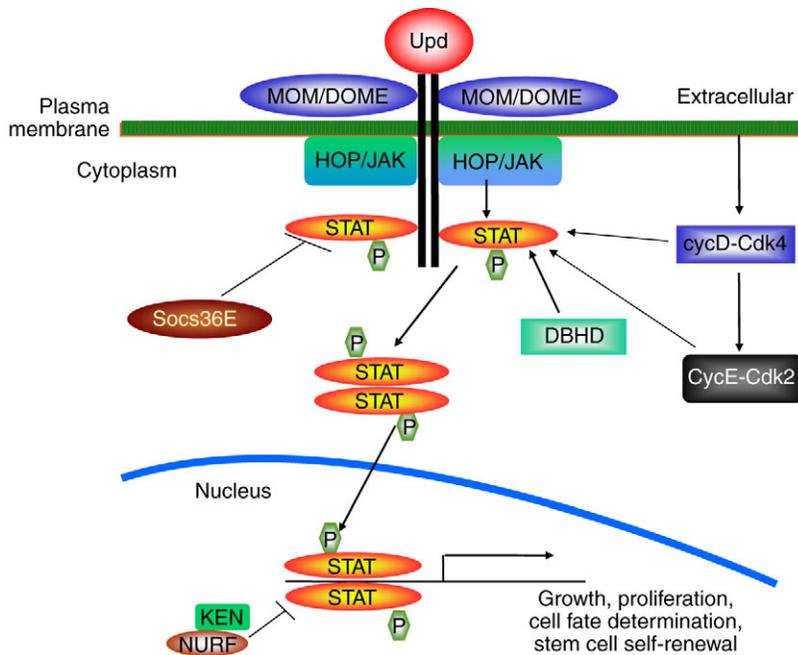


Fig. 3. Schematic diagram of the JAK-STAT pathway. The components of JAK-STAT are: JAK, encoded by the *hopscotch* (*hop*) gene; STAT, encoded by the *Stat92E* (signal-transducer and activator of transcription protein at 92E) gene; a ligand, encoded by the *unpaired* (*upd*) gene; a receptor, encoded by the *domeless* (*dome*)/Master of Marrelle (*mom*) gene; and negative regulators, *Socs36E*, *ken* and *NURF*. The activated Stat92E enters the nucleus to activate its target genes' transcription. Several other molecules interact and regulate the STAT activity, such as cyclin D (*cycD-Cdk4*), cyclin E (*CycE-Cdk2*), and the *Drosophila* homolog of BHD.

are expressed in RNSCs. Using these findings, we proposed that self-renewal of RNSCs is controlled by autocrine JAK-STAT signaling and does not need a fixed niche. This is similar to intestinal stem cells (ISCs) in adult *Drosophila* midgut, where ISCs do not rely on any fixed anatomic niche because ISCs themselves play an active role in supplying signals, such as the Delta ligand, to their daughters to control their proliferation and differentiation (Ohlstein and Spradling, 2006; Ohlstein and Spradling, 2007). However, the RNSCs reside primarily in the lower tubules and ureters, even in Upd-overexpressing flies, suggesting that, in addition

to JAK-STAT signaling, other signaling or molecules restrict the RNSCs to the lower tubules and ureters. Furthermore, in observing the activity of JAK-STAT signaling in MTs by using a Stat92E-GFP reporter, which is expressed in cells with small nuclei between the ureter and the lower part of the main segment, we noticed that some cells with small nuclei strongly express GFP, and others weakly express GFP. These observations suggest that high JAK-STAT signaling regulates RNSC self-renewal, whereas weak JAK-STAT signaling prepares an RB for differentiation into an RC in the lower tubules and ureters, and into a PC or SC in the upper tubules (Fig. 2H). These findings on the MTs of adult *Drosophila* suggest that resident stem cells may be involved in repair and/or regeneration of the renal tissues after acute and/or ischemic injury in the mammalian kidney, and that a fixed niche may not be needed to maintain the self-renewal of renal stem cells (Singh et al., 2007; Affolter and Barde, 2007; Singh and Hou, 2008).

#### Stem cells and kidney cancer

The normal development of the mammalian kidney begins with a reciprocal inductive interaction between the UB and MM that leads to the evolution of the collecting duct system and nephrons, respectively. This process requires a proper sequence and balance of proliferation, cell-cell interactions, apoptosis and differentiation. Any misregulation in these processes may lead to kidney disease, including acute or chronic renal failure, polycystic kidney disease or glomerulonephritis or kidney cancer. Globally, more than 500 million individuals have some form of chronic kidney disease (CKD). Most of these individuals depend on dialysis and rarely receive transplants because of the limited availability of the organ.

Cancer is a leading cause of death worldwide. Cancer begins when a group of cells display uncontrolled growth, invasion and sometimes metastasis. The malignant tumors develop through the accumulation of genetic changes in proliferating cells, such as the activation of oncogenes, dysfunction of stability genes, or inactivation of tumor-suppressor genes. Kidney cancer is a combination of different types of cancer, each with a different histology, requiring different clinical courses and responding to different forms of therapy, and

components are: JAK, encoded by the *hop* (*hopscotch*) gene; STAT, encoded by the *Stat92E* (signal-transducer and activator of transcription protein at 92E) gene; a ligand, encoded by the *upd* (*unpaired*) gene; a receptor, encoded by the *dome* [*domeless*; also known as *mom* (Master of Marrelle)] gene; and several negative regulators such as *suppressor of cytokine signaling at 36E* (*Socs36E*), *ken* and *barbie* (*ken*), *protein inhibitors of activated stats* (*PIAS*), and *nucleosome remodeling factor* (*NURF*) (Luo and Dearolf, 2001; Hou et al., 2002; Agaisse and Perrimon, 2004; Singh et al., 2005; Hombria and Sotillos, 2006; Arbouzova and Zeidler, 2006; Gregory et al., 2008). The activated Stat92E enters the nucleus to activate the transcription of its target genes (Fig. 3). We observed that most of the components of JAK-STAT signaling in MTs is expressed in cells with small nuclei of the lower tubules and ureter region (Fig. 2C,D). They are also sometimes positive for H3P, suggesting that these cells are mitotically active and are expressed in renal stem cell or their immediate daughter RBs.

We also examined the role of JAK-STAT signaling in renal stem cells by using the PMML technique. We found that when we overexpressed the JAK-STAT ligand Upd, the size of the MT increased. We also saw a dramatic increase in the expression of Stat92E, as well as in the number of proliferating cells, mitotically active cells, and renal stem cells, compared with wild-type MTs. These observations suggest that overexpression of Upd makes renal stem cells more active and accelerates cell division of the RNSCs. When we reduced the JAK-STAT signaling by using the PMML technique to overexpress *Socs36E*, the renal stem cells differentiated prematurely. Furthermore, we determined whether JAK-STAT signaling was directly required in renal stem cells. Using the mosaic analysis with the repressible cell marker (MARCM) technique (Lee and Luo, 2001), we analyzed Stat92E homozygous clones in the MTs and found that the absence of JAK-STAT signaling promotes the differentiation of RNSCs, as well as the loss of stem cell population. Furthermore, using Apoptag staining we found that the loss of RNSCs is not due to cell death. Next, we observed the expression pattern of the JAK-STAT ligand Upd and its receptor Dome in the MTs and found that both the ligand and its receptor, along with Stat92E protein,

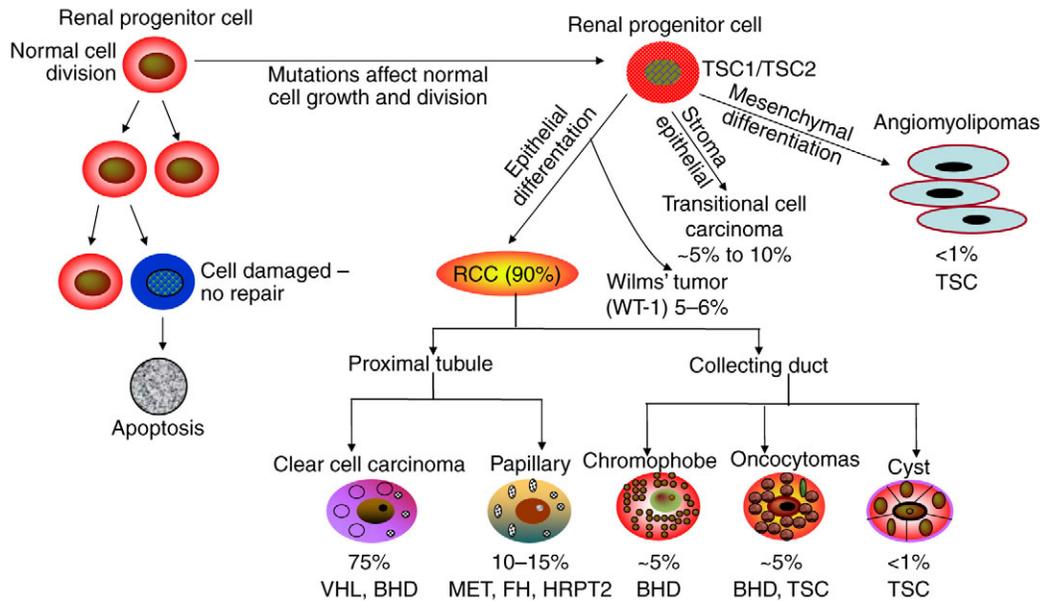


Fig. 4. Schematic summary of genes regulating renal cell carcinoma (RCC) in mammalian kidney. Kidney tissues are made up of many types of cells. These cells grow and divide in a controlled way to produce more cells as they are needed to keep the kidney healthy. When normal cells are damaged beyond repair, they are eliminated by apoptosis. When the genetic material of a cell is altered, cells avoid apoptosis and continue to multiply in an unregulated manner, accumulating mutations that affect normal cell growth and division and resulting in tumor formation. Model shows the renal tumors caused by mutation of the different genes. Renal progenitor cells are regulated by TSCs (tuberous sclerosis complex; TSC1 and TSC2) 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 RCC type. RCC originates primarily in the proximal renal tubules and, rarely, in collecting ducts. These tumors are clear cell carcinoma, papillary, chromophobe or oncocytomas. Some of the tumors are of epithelial origin and are seen, only seen in children (Wilms' tumor); some of the tumors are also caused by the combination of both epithelial and stroma cells (transitional cell carcinomas).

each associated with the alteration of a different gene. Renal cell carcinoma (RCC) is the most common type of kidney cancer, accounting for more than 90% of malignant kidney tumors. RCC originates primarily in proximal renal tubules and, rarely, in collecting ducts (Fig. 4). Like most cancers, RCC is difficult to treat once it has metastasized. The five human genes associated with predisposition to RCC are: von Hippel-Lindau (*VHL*; clear cell RCC); met proto-oncogene (*MET*; papillary RCC); fumarate hydratase (*FH*; papillary RCC); Birt-Hogg-Dubé [*BHD* (also known as *FLCN*); chromophobe oncocytomas, clear cell]; and hyperparathyroidism 2 [*HRPT2* (also known as *CDC73*); papillary RCC; Fig. 4]. RCC could develop following chronic renal regeneration and repair in individuals with polycystic kidney disease or in renal allograft. A detailed investigation of kidney neoplasms suggests that some RCCs, such as Wilms' tumor (WT) and hereditary papillary renal carcinoma, are caused by mutations in the genes involved in normal nephrogenesis. WT is a pediatric kidney cancer that arises from multipotent embryonic renal precursors of the metanephric blastema that fail to differentiate. Some of the neoplasms are caused by mutations in genes expressed during normal development; for example, RCC is associated with the tuberous sclerosis complex (TSC) gene, and clear cell renal carcinoma with the *VHL* gene. The majority of RCCs develop in abnormalities of renal epithelial cells, and only a mutation in *TSC* causes abnormalities in both mesenchymal cells and epithelial cells (Fig. 4) (Henske, 2005; Pfaffenroth and Linehan, 2008).

Studies of the genetic conditions associated with RCC, such as *VHL*, *BHD* and *MET*, as well as genetic analyses of the tumors have provided considerable insight into the pathogenesis of these lesions. Although RCC is resistant to chemotherapy, kinase inhibitors and interleukin-2 are used to treat advanced RCC.

However, because of the side effects, these therapies are not effective. Cancer is considered a stem-cell disease because of both its propagation by a minority of cells with stem-cell-like properties (termed cancer stem cells, or CSCs) and its possible derivation from normal-tissue stem cells (Sneddon and Werb, 2008). Furthermore, overlapping sets of molecules and pathways regulate both stem cell migration and cancer metastasis. Normally, most adult stem cells reside in a quiescent state. However, the effect of repeated injury (as in chronic kidney injury, for example) would, over time, increase the pool size of stem cells in an active state of renewal and increase cancer incidence. The presence of more stem cells in a tissue may enhance the possibility of a stem cell being trapped in the activated state by an oncogenic event (Beachy et al., 2004).

Abnormal functioning of signaling pathways is believed to contribute to the pathogenesis of many malignancies and is particularly relevant to renal cancers. To understand the cancer stem cells in tumors, we need to know first what signaling pathways and pathway interactions regulate cancer formation. It has been shown that the TSC complex [TSC1–TSC2 (also known as hamartin and tuberlin)] regulates early renal progenitor cells because individuals with TSC mutations have an increased incidence of RCC. Mutations in the TSC activate the mammalian target of rapamycin (TOR) by inhibiting RHEB (RAS homolog enriched in brain) and the biochemical phenotypes resemble those of *VHL*. In addition, TOR has been shown to regulate hypoxia inducible factor (HIF) activity, and much evidence implicates TOR as a valid target for treatment of renal cell carcinoma. Epidermal growth factor receptor (EGFR) activates phosphoinositide 3-kinase and downstream targets including AKT (protein kinase B) and TOR, resulting in increased HIF-1 expression leading to tumor metastasis. The *MET* proto-oncogene, expressed in both stem and cancer cells, is a key regulator of invasive growth in normal conditions

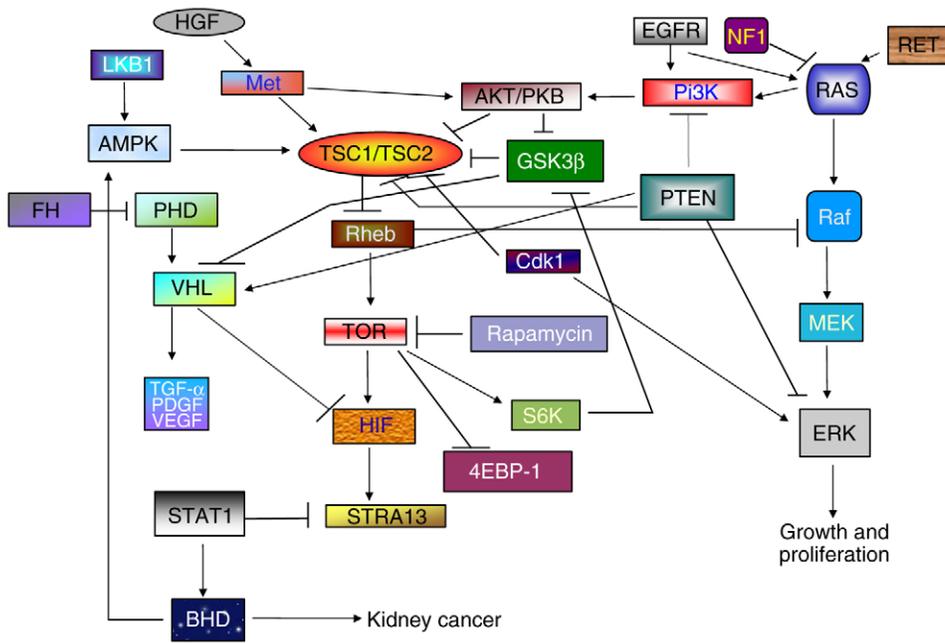


Fig. 5. Proposed model of molecular pathways that regulate kidney tumors in mammals. See text for full names of proteins.

because it binds with HGF to induce receptor dimerization and phosphorylation, and interacts with several other intracellular factors, including the RAS oncogene–mitogen-activated protein kinase (RAS-MAPK) and protein kinase B (AKT) pathways. *VHL*, a tumor-suppressor gene, plays a role in the regulation of tumor angiogenesis by targeting the hypoxia-inducible growth factor 1 (HIF1) for ubiquitin-mediated degradation. HIF1 induces the dedifferentiation of cancer cells, maintains stem cell identity, and increases the metastatic potential (Kondo et al., 2003). *VHL* interacts with HIF1, resulting in the loss of *VHL* and overproduction of the HIF1, which contributes to the development RCC. These observations suggested that the HIF is the potential therapeutic target of *VHL*. Furthermore, genetic inactivation of *VHL* prevents HIF1 down-regulation, leading to the expression of the *MET* proto-oncogene, an important regulator of invasion and metastasis. *VHL* and glycogen synthase kinase 3 (*GSK3*) function together in a ciliary-maintenance signaling network, disruption of which enhances the vulnerability of cells to lose their cilia, thereby promoting cyst formation. Recently it has been reported that *PTEN* (phosphatase and tensin homolog) tumor-suppressor protein cooperates with *VHL* to regulate kidney tumorigenesis (Frew et al., 2008). *STRA13* is a cancer-associated protein regulated by *VHL* and the HIF1 pathway, and is overexpressed in many common malignancies. *VHL* deficiency or HIF1 activation results in the repression of endogenous *STAT1*, which possesses tumor-suppressor properties and is mediated by *STRA13* (Ivanov et al., 2007). *BHD* is another dominantly inherited hamartoma syndrome (Schmidt et al., 2005) that shares several features with *TSC*; mutation in both genes causes renal carcinoma, which suggests that the *BHD* and *TSC* proteins may function within a common pathway. However, clinical phenotypes and the risk of malignancy are higher in *BHD* than in *TSC*. Recently, we demonstrated that amplifying the JAK-*STAT* signaling by overexpressing its ligand *Upd* stimulates the RNSCs to proliferate and differentiate into RCs, which results in tumorous overgrowth in the MT (Singh et al., 2007). Previously, we have reported that *BHD* interacts with JAK-*STAT* signaling and regulates germline stem cell in the *Drosophila* testis and functions downstream of JAK-*STAT* signaling pathways (Singh et al., 2006). Therefore, the *Drosophila* RNSC system may also be a valuable *in vivo* system in

which to study cancer stem cell regulation in renal tubules. The genetic interactions of different oncogenes and tumor-suppressor genes regulating renal carcinoma and other kidney cancers are summarized in Fig. 5. A key goal in clinical oncology is the development of medical therapies specific to pathways that are misregulated in cancer. Understanding the biological pathways involving *VHL*, *MET*, *FH*, *BHD* and *HRPT2* will provide new therapeutic approaches for kidney cancer.

The incidence of end-stage renal disease in patients with chronic kidney disease is predicted to rise in the near future, and probably will not be counterbalanced by currently available renal replacement therapies such as hemodialysis and hemofiltration (Braam et al., 2007). These therapies repair or replace only the filtration function for small solutes and do not replace the lost transport, metabolic and endocrine functions of the kidney. In addition to conventional methods of renal therapy, four innovative ways have been suggested to restore the normal function of the kidney cells after chronic disease or ischemic injury. First, damaged kidneys can be restored by stem cell technology (cell-targeted therapy) and knowledge of developmental programming; second, using therapeutic cloning, a kidney may be grown *in vitro* and transplanted into the recipient; third, other organs may be used to replace various renal functions; and finally, the artificial kidney may be used, which has the potential to be supplemented with human cells (Challen et al., 2006; Braam et al., 2007). These innovative therapies to replace the functions of the kidney, particularly stem cell therapy, may provide invaluable treatments for renal failure. Furthermore, understanding the response to tissue injury, as well as the signals that regulate the activation of tissue stem cells, will provide potential strategies for the use of stem cells in cancer prevention and therapy.

#### Conclusions and future prospects

Regardless of several differences, the general organization and physiology of mammalian kidneys and *Drosophila* MTs show clear similarities in development and function. Both use common strategies of development, and even some of the pathways and molecules are conserved in both systems. Adult stem cells have been well defined to contribute to tissue regeneration after injury in various organ systems.

The recruitment of stem cells to the injured tissue therefore appears to be the prerequisite for repair, and understanding the mechanisms that regulate their migration is crucial for the success of any clinical strategy involving stem cells. Despite the abundant promise of progress, there remains the essential challenge in understanding the role of stem cells in the kidney. Therefore, identifying and characterizing this role is critical to renal research, which must move forward before approaches to therapy and repair, can be fully realized.

Using genetic-labeling techniques and molecular markers, we have identified many multipotent stem cells in the MTs of the adult *Drosophila*. These renal stem cells have the ability to generate all cell types of the adult. Furthermore, we found that autocrine JAK-STAT signaling regulates self-renewal or differentiation of renal stem cells. However, several important questions remain regarding the biological properties of multipotent stem cells in *Drosophila* renal tubules, such as how the self-renewal or differentiation is balanced in the MTs to avoid tissue overgrowth; whether their self-renewal is unlimited; what are the molecular mechanisms controlling asymmetric stem cell division; whether cell polarity, centrosomal asymmetry, and cell cycle regulators, determines asymmetric division of RNSCs, as in other stem cell systems; and finally, how the tumor suppressor genes regulate stem cell proliferation and self-renewal in MTs. Answering these questions is crucial and will provide a better understanding of the mechanisms behind self-renewal, proliferation and differentiation of stem cells in general.

Studies to determine the sources of kidney regeneration after injury and the existence of stem cells and their location have produced conflicting results in the mammalian system, and several experiments are needed to identify the sources and types of cells involved in this process. Furthermore, strategies must be developed to overcome the severity of tubular injury and find a better way to improve the capability of tubular regeneration after acute renal failure. Identification of multipotent stem cells in adult *Drosophila* MTs suggests that resident stem cells may be responsible for repair and regeneration of completely damaged tissues in the mammalian kidney (Humphreys et al., 2008). Understanding the function of multipotent kidney stem cell in adults, combined with powerful, state-of-the-art forward- and reverse-genetic approaches, genomics, and proteomics tools, and studies of the *Drosophila* renal stem cell system, will lead to a better understanding of kidney development. Understanding this process has important clinical implications for early detection, prevention and treatment of several kidney diseases, including cancer.

#### List of abbreviations

CSC	cancer stem cell
CKD	chronic kidney disease
GFP	green fluorescent protein
JAK	Janus kinases
MARCM	mosaic analysis with a repressible cell marker
MET	mesenchymal-to-epithelial transitions
MM	metanephric mesenchyme
MTs	Malpighian tubules
PC	principal cells
PMML	positively marked mosaic lineage
RBs	renalblasts
RC	renalcyte
RCC	renal cell carcinoma
RNSCs	renal and nephric stem cells
SC	stellate cells
SP	side population
STAT	signal transducers and activators of transcription
UB	ureteric bud
WD	Wolffian duct
WT	Wilms' tumor

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