VHL Loss of Function and Its Impact on Oncogenic Signaling Networks in Clear Cell Renal Cell Carcinoma

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Abstract

Loss of *von Hippel-Lindau* tumor suppressor gene function occurs in familial and most sporadic clear cell renal cell carcinoma, resulting in the aberrant expression of genes that control cell proliferation, metabolism, invasion and angiogenesis. The molecular mechanisms by which loss of function leads to tumorigenesis are not yet fully defined. The *von Hippel-Lindau* gene product is part of an ubiquitin ligase complex that targets hypoxia inducible factors for polyubiquitination and proteasomal degradation, linking hypoxia response genes to renal cell carcinoma oncogenesis. Loss *von Hippel-Lindau* gene function also promotes cell invasiveness in response to hepatocyte growth factor, an important regulator of kidney development and renal homeostasis. Increased cell invasiveness is mediated by another ubiquitin ligase target with relevance to the molecular pathogenesis of renal cell carcinoma: β-catenin. This discovery and other recent insights into kidney cancer oncogenesis implicate convergent developmental and homeostatic signaling pathways in tumorigenesis, tumor invasiveness and metastasis.

Keywords

Signalling Network Facts

- Loss of *VHL* gene function occurs in hereditary and sporadic forms of ccRCC

- The *VHL* gene product, pVHL, is part of an oxygen sensor that targets HIFs for degradation

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- pVHL also targets β-catenin for degradation in adults, thereby attenuating developmental responses to HGF and Wnts

- Dysregulated HGF and Wnt signaling also occurs in ccRCC, partly due to pVHL loss

- Further insight into signaling can be found at: http://www.genome.ad.jp/kegg/pathway/hsa/hsa05211.html

Introduction

von Hippel-Lindau (VHL) syndrome is an autosomal dominant hereditary neoplastic disorder characterized by the development of tumors in the cerebellum, spine, retina, inner ear, pancreas, adrenal glands, and kidneys (reviewed in Linehan et al., 2007). VHL-associated bilateral multifocal renal tumors are malignant and metastatic; up to 40% of untreated patients with VHL syndrome have died of advanced clear cell renal cell carcinoma (ccRCC). Affected individuals inherit an altered copy of the *VHL* tumor suppressor gene, located on chromosome 3 (3p25-26), and the remaining wild type copy is later inactivated in somatic cells, most often by loss of chromosome 3p or *VHL* gene hypermethylation. Alterations or deletions of 3p also occur in the majority of sporadic ccRCC cases, which claims more than 13,000 lives each year in the U.S. alone (Linehan et al., 2007). Reconstitution of wild type *VHL* expression in ccRCC-derived cell lines has been shown to regulate tumorigenesis in athymic nude mice, confirming a fundamental role for *VHL* in ccRCC oncogenesis (reviewed in Kaelin, 2002, Linehan et al., 2007).

Functions

Biochemical studies of the protein encoded by *VHL* (pVHL) have revealed that it forms a stable complex with other proteins possessing E3 ubiquitin ligase activity. This complex is best known for targeting hypoxia inducible factors (HIFs) for polyubiquitination and subsequent proteasomal degradation (Kaelin, 2002). HIF family transcription factors control the expression of genes involved in the cellular response to hypoxia. HIFs are constitutively expressed and under normoxic conditions, protein levels, and thus activity, are continuously suppressed by pVHL. Under hypoxic conditions or when the *VHL* gene is mutated or lost, HIFs accumulate, leading to the increased expression of genes encoding vascular endothelial growth factor, platelet derived growth factor, transforming growth factor-α, erythropoietin, the hepatocyte growth factor (HGF) receptor, Met, and others, all of which are potentially important in RCC oncogenesis (Kaelin, 2002, Linehan et al., 2007). Cultured *VHL*-negative ccRCC cells contain elevated levels of HIFs and, unlike normal, fully differentiated renal epithelial cells, respond to HGF treatment with increased motility, matrix invasion and morphogenesis (Peruzzi et al., 2006). These HGF responses are abolished when wild type *VHL* expression is reconstituted in ccRCC cells, directly linking loss of *VHL* function to an invasive tumor phenotype (Peruzzi et al., 2006).

The mechanism by which pVHL represses HGF-driven ccRCC cell invasiveness was elucidated by Peruzzi et al. (2006), who hypothesized that pVHL negatively regulates Met-driven β-catenin signaling in mature renal tubule epithelial cells. β-catenin normally maintains adherens junctions and, in response to HGF or Wnt stimulation, accumulates in the cytoplasm and translocates to the nucleus where it associates with T-cell factor/lymphoid enhancer factor (TCF/LEF) family members to function as a transcriptional co-activator (Clevers, 2006). Consistent with a shift in function from adhesion to signaling, Peruzzi et al. (2006) found that HGF stimulated the redistribution of β-catenin from peripheral to cytoplasmic and nuclear pools in *VHL*-negative ccRCC cells and that restoration of pVHL production repressed HGF-stimulated β-catenin target gene activation. Moreover, ectopic expression of a dominant-negative form of TCF blocked the invasive HGF response of pVHL-negative ccRCC cells

(Peruzzi et al., 2006). In addition to revealing an important novel target for pVHL, these findings identified β-catenin as a potential target for biomarker and therapeutic development in ccRCC.

Cascades

HGF signaling between mesenchymal and epithelial cells is a driving force in kidney morphogenesis and differentiation, and inappropriate Met signaling in cancer can resemble HGF-regulated developmental transitions between epithelial and mesenchymal cell types (Birchmeier et al., 2003). β-catenin is likely to mediate both HGF and Wnt signaling during nephrogenesis (Perantoni, 2003); relevant aspects of these convergent networks are schematically depicted in Figure 1.

Like HGF, Wnts act broadly in embryogenesis and adulthood, including kidney development and homeostasis (Dressler, 2006). Aberrant Wnt/β-catenin signaling occurs in many cancers, particularly colorectal cancer (Clevers, 2006). Although activation of the pathway in cancer is usually attributed to deregulation of downstream effectors (e.g. β-catenin) or suppressors (adenomatous polyposis coli protein or Axin), autocrine mechanisms may also be involved (Rubin et al., 2006). All of these signaling events are mediated by Wnt receptors in the Frizzled (Fzd) family and the low density lipoprotein (LDL) receptor-related proteins 5 and 6 (LRP5/6) co-receptors. Wnt-stimulated Fzd/LRP activation inhibits the otherwise constitutive degradation of cytosolic β-catenin, permitting nuclear translocation, TCF/LEF binding and the activation of genes controlling cell proliferation, migration and morphogenesis (Clevers, 2006). Unlike HGF signaling, which is primarily paracrine, normal Wnt signaling is frequently autocrine. Wnt signaling is also regulated at the cell surface by secreted Frizzled-related proteins (sFRPs), which bind Wnts and typically antagonize Wnt signaling, and Dickkopfs (DKKs), which bind LRP5/6 and block Wnt-dependent β-catenin transcriptional activity (Rubin et al., 2006). It is widely thought that negative regulation of autocrine Wnt signaling by sFRPs and DKKs is likely to be important in both developmental and homeostatic contexts.

Consistent with the known role of HGF in regulating developmental transitions between epithelial and mesenchymal cell types, β-catenin and E-cadherin, another adherens junction protein, are upregulated early in kidney development upon transition of the mesenchyme surrounding the branching ureteric buds into the epithelium that will form the tubules of the nephron (Huber et al., 2000). This process and the ensuing tubule formation involves several Wnt family members acting in an autocrine manner (Perantoni, 2003), as well as HGF acting in a paracrine mode (van Adelsberg et al., 2001). The upregulation of *VHL* late in nephrogenesis, and its persistent expression throughout adulthood, is thought to be critical in attenuating HIF-mediated proliferation and morphogenesis in mature renal epithelial cells. *VHL* and *HIF* genes display reciprocal temporal expression patterns during renal development that point to a role for hypoxia in driving early nephrogenesis and for pVHL in limiting this process (Bernhardt et al., 2006, Richards et al., 1996). For example, *VHL* mRNA has been found in mid-phase (mesonephric) duct epithelia and tubules, but not in the early part of final (metanephric) development (Richards et al., 1996). Later, *VHL* expression re-emerges throughout the tubular epithelium of the metanephric kidney (Richards et al., 1996). In contrast, marked nuclear localization of HIF-1α has been found in early medullary and cortical collecting ducts and in glomerular cells, whereas HIF-2α is reportedly produced in interstitial and peritubular cells and podocytes of the more mature glomeruli, consistent with roles in tubulogenesis and renal vasculogenesis, respectively (Bernhardt et al., 2006). Upon completion of kidney development, HIF-1α and -2α proteins are no longer detected (Bernhardt et al., 2006), consistent with proteasomal targeting by upregulated pVHL expression.

The recently described convergence of pVHL, HGF and β-catenin pathways suggests that *VHL* upregulation also may be important for attenuating β-catenin-mediated phenotypic changes once kidney development is completed. *HGF* and *MET* expression persist in the normal adult kidney, but the response of renal epithelial cells to HGF stimulation undergoes striking changes upon completion of normal development. Morphogenic and proliferative responses are minimized, and HGF signaling in the adult becomes adapted for renal homeostasis (Liu, 2006), protecting adult kidney tissue from toxicity and ischemic stress (Matsumoto and Nakamura, 2001) and counteracting fibrosis, a major cause of renal failure (Liu, 2006). This change in response coincides with tight control of HIF protein levels in the adult kidney by pVHL. When *VHL* function is lost, the resulting combined derepression of HIFs and β-catenin is likely to contribute to acquisition of the malignant ccRCC phenotype, resembling the aberrant activation of a robust developmental signaling program.

Key Molecules

As inhibitors of autocrine Wnt signaling, sFRPs have been viewed as potential tumor suppressors. Consistent with this hypothesis, the chromosomal loci of *sFRP*s have been associated with loss of heterozygosity in various tumor types and loss of *SFRP* expression in cancer due to promoter hypermethylation is well documented (Rubin et al., 2006). Loss of *SFRP1* expression in breast cancer correlated with decreased survival and restoration of expression in colorectal tumor cell lines resulted in an attenuated tumor phenotype (Rubin et al., 2006). Interestingly, the attenuated phenotype was observed in cells with mutations in APC or β-catenin, providing support for the concept that Wnt stimulation is needed to drive oncogenic signaling by these mutations. Reduced expression of *DKKs* also has been reported for various tumors (Rubin et al., 2006). Kurose et al. (2004) reported a high incidence of *DKK-3* silencing specifically in RCC, suggesting that DKKs also possess tumor suppressor activity. A recent and comprehensive review of Wnt signaling in renal cancer is available (Guillen-Ahlers, 2008).

Recently, Dalh et al. (2007) and Gumz et al. (2007) reported a remarkably high incidence of *SFRP1* loss in ccRCC. In addition, Wnt responsive genes were found to be dramatically upregulated in ccRCC specimens (Gumz et al., 2007). Restoration of *SFRP1* expression in ccRCC cell lines decreased the expression of these genes by two- to threefold (Gumz et al., 2007). *SFRP1* expressing ccRCC cell lines also displayed dramatically reduced growth in culture and in soft agar (Gumz et al., 2007). Finally, stable transfection of three clonal ccRCC cell lines with *SFRP1* cDNA expression constructs resulted in complete blockade of tumor xenograft growth in mice (Gumz et al., 2007). These results provide strong evidence that loss of *SFRP1* expression is a key event in ccRCC tumorigenesis, and proof of concept that *SFRP1* restoration is a potential ccRCC treatment modality.

The convergence of dysregulated HIF, HGF and Wnt/β-catenin signaling networks in ccRCC may amplify their individual oncogenic effects (Figure 2). Loss of E-cadherin expression occurs in many tumor types, including ccRCC, and is associated with increased β-catenin transcriptional activity and the acquisition of an invasive cell phenotype (Russell and Ohh, 2007). Concomitant stimulation by HGF leads to Met-mediated tyrosyl phosphorylation of β-catenin, reducing its affinity for junctional E-cadherin and promoting its binding to Bcl9-2 (Brembeck et al., 2004). As a result, adherens junctions are further disrupted and cytoplasmic β-catenin is more efficiently translocated to the nucleus, enhancing cell invasiveness (Peruzzi et al., 2006). Dysregulated activation of HIF target genes, promoting cell survival, proliferation, motility, matrix remodeling and angiogenesis, compounds aberrant HGF and Wnt signaling (Kaelin, 2002,Linehan et al., 2007,Peruzzi et al., 2006). Finally, as reported by Kaidi et al. (2007), HIF-1α directly modulates β-catenin-dependent gene expression by competing with TCF/LEF proteins for binding to nuclear β-catenin. Moreover, β-catenin-HIF-1α interaction

enhances HIF mediated transcription (Kaidi et al., 2007). In light of the many levels at which these pathways coincide, it is tempting to speculate that partial loss of *VHL* function that precedes inactivation of both alleles, combined with modest activity in the HGF and Wnt/β-catenin pathways, may, in the absence of other genetic defects, drive renal epithelial cells toward tumorigenesis.

Associated Pathologies and Therapeutic Implications

Despite recent regulatory approval of two new drugs to treat ccRCC, there is no broadly effective, durable therapy for this disease once it becomes metastatic. While imaging techniques have improved the detection of localized ccRCC, these tumors often are asymptomatic until they have spread systemically, and patients that present with advanced disease have only an 18% two-year survival rate (Linehan et al., 2007). Consequently, early detection methods and new therapies are urgently needed. Recent progress in defining ccRCC cell invasiveness mediated by HGF, HIF and Wnt driven β-catenin signaling identifies each of these molecules, and HIF-β-catenin interaction, as potential targets for drug development. The identification of *SFRP* and *DKK* gene hypermethylation as a frequent feature of ccRCC, and the demonstration that restored *SFRP1* expression can block ccRCC tumorigenesis in animals, suggest that demethylating agents or methylase inhibitors that could upregulate *SFRP1* expression are also potential ccRCC treatment strategies. Finally, analysis of *SFRP* gene hypermethylation may have diagnostic and prognostic value in the detection and clinical management of ccRCC (Urakami et al., 2006). Continued research into the molecular pathogenesis of ccRCC will better define this collection of critical oncogenic events and facilitate the development of combination therapies that ultimately may be needed for the effective treatment of this and other human cancers.

Acknowledgments

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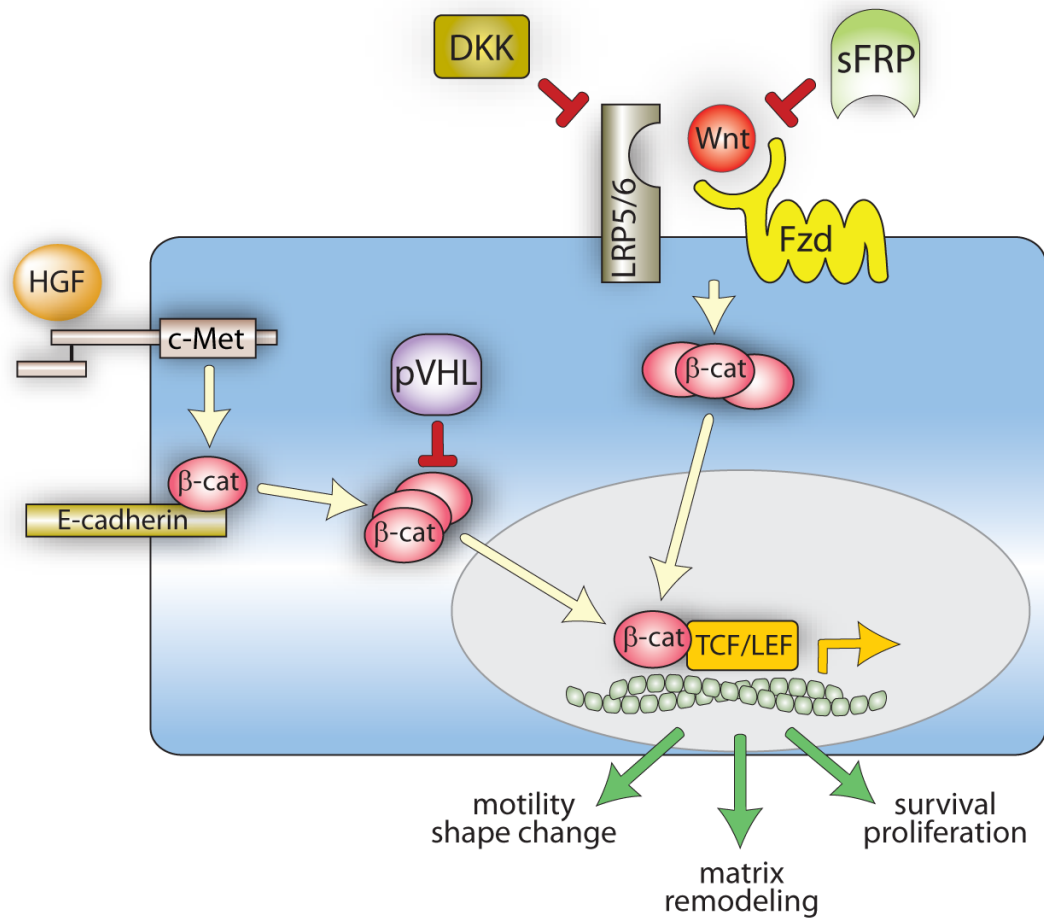**Figure 1. Hypothetical model of HGF and Wnt signaling in kidney development and adulthood**
During development, HGF-Met binding promotes receptor kinase activation and tyrosyl phosphorylation of junctional β-catenin, resulting in its dissociation from E-cadherin and cytoplasmic accumulation. Wnt binding to Fzd and LRP5/6 induces Fzd activation, which stabilizes cytoplasmic β-catenin by protecting it from constitutive degradation. Cytoplasmic β-catenin from either pathway translocates to the nucleus, where it associates with members of TCF/LEF transcription factor family and promotes the expression of genes controlling cell motility, proliferation and matrix remodeling. In adult, fully differentiated renal epithelial cells, pVHL targets cytosolic β-catenin for degradation and balanced expression of Wnts and the negative regulators DKKs and sFRPs keeps Fzd activation, and thus cytosolic β-catenin, to a minimum.
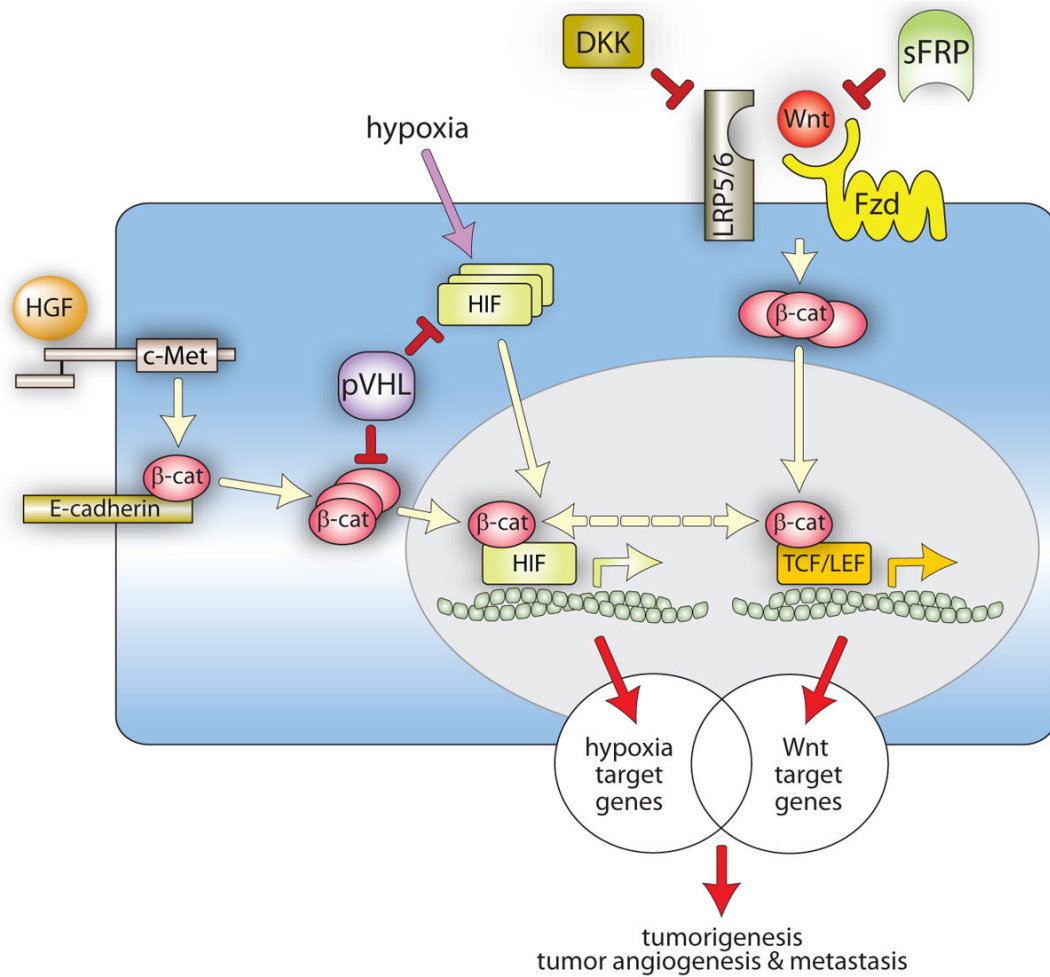
Figure 2. Convergence of HIF and β-catenin signaling networks at multiple levels in ccRCC
Wnt-Fzd-LRP5/6 and HGF-Met interactions promote increases in cytosolic β-catenin normally kept in check by sFRPs, DKKs and pVHL. The loss of these negative regulators in ccRCC, as well as hypoxia during tumor progression, results in the aberrant accumulation of cytoplasmic and nuclear β-catenin and HIF through multiple pathways, with compounding effects on target gene expression. Nuclear β-catenin interacts either with HIF or TCF/LEF to promote the expression of overlapping sets of genes, contributing to tumorigenesis, tumor angiogenesis and metastasis.