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## The genetic basis of kidney cancer: a metabolic disease

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### Abstract

Kidney cancer is not a single disease but encompasses a number of different types of cancer that occur in the kidney, each caused by a different gene with a different histology and clinical course that responds differently to therapy. Each of the seven known kidney cancer genes, *VHL*, *MET*, *FLCN*, *TSC1*, *TSC2*, *FH* and *SDH*, is involved in pathways that respond to metabolic stress and/or nutrient stimulation. The VHL protein is a component of the oxygen and iron sensing pathway that regulates HIF levels in the cell. HGF/MET signaling affects the LKB1/AMPK energy sensing cascade. The FLCN/FNIP1/FNIP2 complex binds AMPK and therefore may interact with the cellular energy and nutrient sensing pathways, AMPK-TSC1/2-mTOR and PI3K-Akt-mTOR. TSC1/TSC2 are downstream of AMPK and negatively regulate mTOR in response to cellular energy deficit. FH and SDH play a central role in the mitochondrial tricarboxylic acid (TCA) cycle whose activities are coupled to energy production through oxidative phosphorylation. Mutations in each of these kidney cancer genes result in dysregulation of metabolic pathways involved in oxygen, iron, energy and/or nutrient sensing suggesting that kidney cancer is a disease of cell metabolism. Targeting the fundamental metabolic abnormalities in kidney cancer provides a unique opportunity for the development of more effective forms of therapy for this disease.

### Introduction

Kidney cancer is not a single disease; it comprises a number of different cancers that occur in the kidney, each with a different histology and clinical course, which respond differently to therapy and are caused by mutations in different genes.(1) The study of hereditary kidney cancer syndromes has led to the identification of genes implicated in familial clear cell renal carcinoma, familial chromophobe kidney cancer, familial type 1 and type 2 papillary kidney cancer, familial nonsyndromic renal carcinoma and tuberous sclerosis complex. All cancer is essentially genetic(2) and recently Thompson, et al. have elucidated the central importance of metabolic pathways in cancer.(3–5) Each of the kidney cancer genes identified so far interact with cell metabolism pathways involved in energy, nutrient, iron and/or oxygen sensing (Figure 1).

The *VHL* gene pathway is involved in oxygen and energy sensing. The VHL complex targets the hypoxia inducible factors (HIF) for ubiquitin mediated degradation. This is an oxygen and iron sensing mechanism; when the cell is low in oxygen or iron, the VHL complex cannot degrade HIF and HIF over-accumulates. HGF/MET signaling occurs through both the PI3K/

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Akt/mTOR and the LKB1/AMPK pathways. FLCN, through its interacting partners FNIP1 and FNIP2, binds the bioenergetic sensor, AMP-activated protein kinase (AMPK). In response to energy deficit in the cell, AMPK phosphorylates TSC2 which then complexes with TSC1 and negatively regulates the mTOR pathway. Alterations in any of these genes can affect the energy sensing signaling pathways in the cell.

Hypoxia-inducible factors (HIFs) are oxygen-sensitive basic helix-loop-helix transcription factors, which regulate biological processes that facilitate both oxygen delivery and cellular adaptation to oxygen deprivation. HIF- $\alpha$ , together with the constitutively expressed HIF- $\beta$  subunit, bind to hypoxia-response elements (HRE) in gene promoters to regulate the expression of genes that are involved in energy metabolism, angiogenesis, erythropoiesis, iron metabolism, cell proliferation, apoptosis and other biological processes. HIF1- $\alpha$  and HIF2- $\alpha$  mediate transcription of a number of downstream genes thought to be important in cancer including transforming growth factor alpha (*TGF $\alpha$* ), platelet derived growth factor (*PDGF*), and vascular endothelial growth factor (*VEGF*). We propose that targeting the metabolic defects in this pathway may provide an opportunity for the development of kidney cancer gene specific therapies that are more durable and more effective than existing treatments. The current targeted therapies for *VHL*  $-/-$  clear cell carcinoma have focused on targeting the genes transcriptionally upregulated by HIF such as vascular endothelial growth factor  $\alpha$  (*VEGF $\alpha$* ), vascular endothelial growth factor receptors (*VEGFR*), the platelet derived growth factor receptor (*PDGFR*) or the mTOR/HIF pathway. While most of these agents induce responses in patients with advanced kidney cancer, the responses are usually partial and most patients eventually progress. The fundamental metabolic aspects of these cancer genes may be the Achilles heel that could potentially be exploited to develop more durable and effective forms of therapy. Here we describe the genes that have been identified in clear cell and non-clear-cell kidney cancers, their involvement in cell metabolism pathways and how they might be targeted to provide future therapies.

### **VHL: von Hippel–Lindau**

Positional cloning was used in patients with von Hippel–Lindau to identify the *VHL* gene locus on the short arm of chromosome 3. (6–12) von Hippel–Lindau is a hereditary cancer syndrome; affected individuals are at risk for the development of renal cysts and clear cell kidney cancer. Germline mutations of the *VHL* gene (intragenic, deletions or splicing defects) have been found in almost all families with von Hippel–Lindau, (13) and somatic inactivation of *VHL* by methylation or mutation has been reported in up to 91% of patients with sporadic clear cell renal carcinoma. (14–19)

The VHL protein forms a complex with elongin B, elongin C, and cullin 2(19) that targets HIF for ubiquitin-mediated degradation. (20–22) This process is oxygen-dependent. During normoxia, HIF- $\alpha$  is hydroxylated on a critical proline residue by HIF prolyl hydroxylase (PHD); this process requires molecular oxygen, 2-oxoglutarate, ascorbate and Fe<sup>2+</sup> as cofactors, facilitating HIF- $\alpha$  binding to pVHL, and proteasomal degradation by the E3 ubiquitin ligase complex. Under hypoxic conditions, PHD cannot hydroxylate HIF- $\alpha$ , therefore the pVHL/elonginC/elonginB/cullin complex does not bind HIF and HIF- $\alpha$  accumulates. (21;23) (Figure 2) Similarly, when the *VHL* gene is mutated, mutant pVHL can no longer bind to HIF- $\alpha$  resulting in HIF over accumulation. Although there is considerable overlap in the genes that are transcriptionally regulated by HIF1- $\alpha$  and HIF2- $\alpha$ , *in vitro* and *in vivo* studies have indicated that HIF2- $\alpha$  is the critical HIF for tumorigenesis in clear cell kidney cancer. (24–26) Clear cell kidney cancers (with or without *VHL* mutation) that express both HIF1- $\alpha$  and HIF2- $\alpha$  exhibit enhanced signaling via the mitogen-activated protein kinase (MAPK) and serine/threonine-protein kinase mammalian target of rapamycin (mTOR) pathways, whereas clear cell tumors that express only HIF2- $\alpha$  have elevated c-Myc activity. (27) If HIF2- $\alpha$  is the

critical pathway for kidney cancer tumorigenesis, targeting the HIF2- $\alpha$  pathway would likely be more successful than developing agents which solely target the HIF1- $\alpha$  pathway. (Figure 3) Efforts are currently underway to identify agents which downregulate HIF2- $\alpha$ . An increased understanding of VHL signaling has provided the foundation for the development of therapeutic approaches that target this pathway in patients with advanced clear cell kidney cancer. A number of drugs have recently been approved by the FDA that target either the downstream targets of HIF activity (such as the VEGF receptor) or inhibit mTORC1, which provides translational control of HIF1- $\alpha$ . (28) These agents are discussed in detail elsewhere (Karakiewicz Review, June issue). Briefly, bevacizumab is an antibody that targets VEGFA. Sorafenib is a small molecule multikinase inhibitor that targets VEGF and PDGF receptors and the Raf pathway. (29) Sunitinib also inhibits VEGF and PDGF receptors, and is most frequently used as a first-line agent in patients with advanced clear cell kidney cancer. (30) Axitinib and pazopanib are recently developed selective inhibitors of the VEGF receptors. (31;32) Pazopanib has been approved by the US FDA for treatment of metastatic kidney cancer, while axitinib is currently being evaluated in randomized phase III trials. Early clinical experience with the selective VEGF receptor antagonists suggests that these agents may be better tolerated than sunitinib and sorafenib without compromising efficacy; if these observations are confirmed in phase III randomized trials, selective VEGF receptor antagonists are likely to be increasingly used as first-line agents in patients with advanced clear cell kidney cancer. Temsirolimus and everolimus inhibit mTORC1. Temsirolimus has been shown to prolong survival in previously untreated kidney cancer patients with high-risk features compared to interferon; in patients who have progressed on sunitinib or sorafenib, everolimus is associated with a modest prolongation in progression-free survival compared to placebo and this agent is a reasonable second-line options in patients with advanced kidney cancer who have progressed on front-line therapy targeting the VEGF pathway.

Most of the therapeutic agents currently approved to treat clear cell kidney cancer target selected downstream components of the HIF pathway including VEGF and the VEGF and PDGF receptors. The responses to these agents observed in patients with advanced clear cell kidney cancer is proof of principle that targeting the VHL/HIF pathway can induce tumor regression in humans. However, these agents target only a small portion of the downstream genes regulated by HIF. An approach to target HIF transcriptional activity or translation of HIF itself, i.e. to affect all of the genes regulated by HIF, could potentially provide a more effective approach to therapy. Topotecan is a topoisomerase 1 inhibitor that represses HIF1- $\alpha$ -dependent transcription. (33;34) This agent is currently under evaluation in a phase 1 clinical trial in patients whose tumors express HIF1- $\alpha$  (Clinicaltrials.org; 888-NCI-1937). A number of groups are currently screening for agents that target transcription of HIF2- $\alpha$  transcriptionally regulated genes. (35) mTOR is part of two distinct signaling complexes. The rapamycin-sensitive mTORC1 complex regulates cell growth and protein synthesis in response to growth factor stimulation by phosphorylating S6 kinase and the eIF-4E binding protein to modulate key regulators of mRNA translation. The rapamycin-insensitive complex mTORC2 regulates actin cytoskeleton organization through phosphorylation of protein kinase C $\alpha$  and also phosphorylates Akt at S473 to activate the Akt-mTORC1 pathway. The mTORC1 inhibitor temsirolimus and other 'rapalogs' suppress tumor growth in *VHL*-deficient clear cell kidney cancers via inhibition of HIF-1 $\alpha$  translation. (36) Most *VHL*-deficient tumors, however, express both HIF-1 $\alpha$  and HIF-2 $\alpha$ . (27) Knockout experiments have revealed that HIF1- $\alpha$  translation is dependent on mTORC1 signaling, whereas HIF2- $\alpha$  is downstream of the mTORC2 pathway. (37) New agents that target both the mTORC1 and mTORC2 pathways have the potential to downregulate both HIF1- $\alpha$  and HIF2- $\alpha$  in clear cell kidney cancers and could provide more antitumor activity than temsirolimus and everolimus, which primarily target the mTORC1 pathway. An agent which inhibits both the mTORC1 and mTORC2 pathway, AZD8055, has been recently shown to have potent anti-tumor activity in *in vitro* and *in vivo* model systems

(38) and it is anticipated that clinical trials evaluating the effectiveness of this agent will be initiated soon.

The development of other strategies that decrease translation of HIF may also have significant potential for patients with advanced kidney cancer. The presence of an iron responsive element (IRE) in the 5' untranslated region of *HIF2- $\alpha$*  messenger RNA (mRNA) suggests that components of the iron metabolism pathway might be exploited to this end. (39) Cytoplasmic aconitate hydratase (IRP1) is an iron sensing protein involved in maintaining iron homeostasis. IRP1 binds to the iron responsive elements of *HIF2- $\alpha$*  mRNA and inhibits its translation. (40;41) Overexpression of IRP1 suppresses growth of lung tumor xenografts, (42) which has prompted the search for small molecules that could decrease *HIF2- $\alpha$*  translation by enhancing the binding of IRP1 to the 5' untranslated region of its mRNA. (43) Identification of agents which enhance the binding of IRP1 to the IRE in the 5' untranslated region of the *HIF2- $\alpha$*  mRNA or mimic IRP1 activity could provide a novel targeted approach to therapy.

Turcotte *et al.* (44) have utilized a novel approach to identify agents that target the VHL pathway. They performed a synthetic lethal screen in a set of *VHL*-negative versus *VHL*-positive clear cell kidney cancer cell lines and identified STF-62247, a cytotoxic agent that inhibits tumor growth. STF-62247 selectively induced toxicity in *VHL*  $-/-$  cells in a HIF-independent fashion and was found to induce autophagy. This agent was found to have activity in both *in vitro* and *in vivo* models, suggesting possible therapeutic potential for this or a related agent in humans with advanced kidney cancer. By targeting autophagy, a central component of the cell's response to nutrient and energy deprivation, Turcotte and colleagues have developed a new paradigm for cancer therapy. Many chemotherapeutic and targeted tyrosine kinase approaches are limited by their toxicity to non-tumor cells. However, targeting autophagy was found to affect only the *VHL*  $-/-$  cells whereas cells with a wild-type copy of the *VHL* gene were not affected. (44) This approach, targeting the fundamental metabolic defect in the cell, has the potential to provide a less toxic and more effective form of therapy for patients with this disease.

### ***MET*: Hereditary Papillary Renal Cell Carcinoma**

The proto-oncogene *MET* (hepatocyte growth factor receptor) was identified as the gene for hereditary papillary renal carcinoma by genetic linkage analysis in families with this inherited renal cancer syndrome. *MET* encodes the cell surface receptor for hepatocyte growth factor (HGF), which is involved in mitogenesis, morphogenesis and motogenesis. (45) Activating mutations in the tyrosine kinase domain of *MET*, have been detected in the germline of affected patients and in a subset of sporadic type 1 papillary kidney cancers. (46;47) The histological patterns of hereditary and sporadic type 1 papillary kidney tumors with *MET* mutations share a distinct morphological phenotype consisting of papillary or tubulo/papillary architecture with slender short papillae containing delicate fibrovascular cores lined by small cells with low grade basophilic nuclei and scant amphophilic cytoplasm. (48)

One effect of growth factor-dependent activation of the phosphatidylinositol 3-kinase (PI3K) signaling pathway is increased cell surface expression of nutrient transporters increasing uptake of amino acids, glucose and other nutrients. (4) Nutrient-stimulated HGF/*MET* signaling induces phosphorylation of serine/threonine-protein kinase 11 [STK11; also referred to as LKB1, the upstream kinase of 5' AMP-activated protein kinase (AMPK)] on Ser428 through the RAS-Erk1/2-p90<sup>RSK</sup> pathway, implicating *MET* in the LKB1-AMPK-mTOR nutrient and energy sensing pathway.

A clinical trial is currently underway to determine the effect of foretinib, a kinase inhibitor of both *MET* and *VEGF* receptors, in patients with papillary kidney cancer (hereditary and sporadic). There is early evidence of efficacy of this agent in patients with germline mutations

in the tyrosine kinase domain of *MET*. (49) It is possible that there would be response to an agent which has activity against *MET* in tumors that are characterized by a mutation in the tyrosine kinase domain of *MET*; it is also possible that such an agent would have activity in tumors which have *MET* amplification. It is not known if an agent such as foretinib would have activity against kidney tumors that are caused by mutation of other genes such as TSC1 or TSC2.

### **TSC1/TSC2: Tuberous Sclerosis Complex**

The tuberous sclerosis genes *TSC1* and *TSC2* are also involved in the AMPK/mTOR nutrient and energy sensing pathway (Figure 1). Germline mutations in one or other of these genes have been identified in patients with tuberous sclerosis complex, an autosomal dominant disorder associated with the development of cutaneous angiofibromas, pulmonary lymphangiomyomatosis and renal tumors. (50) Angiomyolipoma is the most common renal tumor found in affected individuals; however, other tumor types have been reported, including clear cell renal carcinoma. (51)

*TSC1* encodes hamartin and *TSC2* encodes tuberin, which form a heterodimer that acts as a GTPase-activating protein toward Rheb, a Ras-family GTPase that activates mTORC1. GTPase activity of the TSC1/2 complex on Rheb results in inhibition of mTOR activity. *TSC1* or *TSC2* deficient tumors exhibit increased phosphorylation of p70S6 kinase, S6 ribosomal protein and 4E-BP1, downstream effectors of mTORC1 activation, and readouts for initiation of mRNA translation and protein synthesis. (50) Lack of TSC1/2 inhibition would presumably also result in HIF accumulation through increased *HIF* mRNA translation by activated mTORC1.

Sirolimus has been demonstrated to cause regression of renal angiomyolipomas in patients with tuberous sclerosis complex. Sirolimus forms a complex with FK binding protein 12 and inhibits mTORC1 signaling. (47) Although most of the kidney tumors that regressed on therapy tended to increase in volume after the therapy was stopped, this study provided the foundation for a molecular therapeutic approach for the treatment of renal tumors associated with the *TSC1-TSC2* pathway. There are currently a number of trials evaluating the role of sirolimus in patients with TSC. When the role of this agent in TSC patients has been defined, future trials that combine this agent with other agents targeting this pathway will be possible.

### **Folliculin: Birt-Hogg-Dubé syndrome**

Genetic linkage analysis in patients with Birt-Hogg-Dubé syndrome identified *folliculin* (*FLCN*), a novel gene on the short arm of chromosome 17, as the causal gene. (52;53) Patients with Birt-Hogg-Dubé syndrome are at risk for the development of benign cutaneous tumors (fibrofolliculomas), (54) pulmonary cysts (54) and chromophobe kidney tumors. (55) (Figure 4) These tumors may be bilateral, multifocal and can metastasize. (56) *FLCN* gene mutations have been detected in over 90% of families affected by Birt-Hogg-Dubé syndrome. (53–59) Most germline mutations that have been detected in *FLCN* are predicted to truncate the protein. The results of DNA sequencing to identify somatic mutations in the wild type allele of *FLCN* in renal tumors from patients with Birt-Hogg-Dubé syndrome revealed loss of heterozygosity of the *FLCN* locus in 17% of renal tumors and sequence alterations in 53%. (60) These findings support the notion that *FLCN* is a tumor suppressor gene.

Folliculin is also involved in the AMPK/mTOR pathway; FLCN forms a complex with folliculin interacting proteins 1 and 2 (FNIP1 and FNIP2), which binds the  $\gamma$ -subunit of AMPK (Figure 5A). (61–63) The functional significance of this interaction and the mechanism by which folliculin may regulate AMPK-mTOR signaling is currently unknown but is under intense investigation by a number of laboratories. One approach to understanding folliculin



function is through the study of *in vivo* animal models in which *FLCN* is inactivated. Both the mTORC1 and mTORC2 pathways were found to be activated in the kidney tumors of a *FLCN* knockout mouse. (64) (Figure 5B) These findings suggest that targeting both the mTORC1 and mTORC2 pathways may show promise for patients with Birt-Hogg-Dubé-associated kidney cancers.

The *FLCN* gene has been selectively inactivated in the kidneys of a murine model; affected mice developed enlarged polycystic kidneys and died from renal failure by 3 weeks of age. (65;66) *FLCN* knockout mice treated with rapamycin had smaller, less cystic kidneys than untreated *FLCN* knockout mice and a longer median survival time. (65) The results in this *in vivo* model of BHD would suggest that rapamycin analogues such as sirolimus might be potential therapeutic agents against Birt-Hogg-Dubé-associated renal tumors.

### Tricarboxylic Acid Cycle Genes

The tricarboxylic acid (TCA) cycle is part of a metabolic pathway coupled to mitochondrial oxidative phosphorylation that converts nutrients to energy in aerobic cells. The fumarate hydratase (*FH*) and succinate dehydrogenase (*SDH*) genes encode mitochondrial TCA cycle enzymes that play an essential role in energy production in the TCA, or Krebs, cycle by catalyzing the conversion of fumarate to malate and succinate to fumarate respectively. Individuals who harbor germline mutations in either of these TCA cycle enzymes have an increased risk for developing renal tumors.

### Fumarate Hydratase: Hereditary Leiomyomatosis Renal Cell Carcinoma

*Fumarate hydratase (FH)* was identified as the gene responsible for hereditary leiomyomatosis renal cell carcinoma (HLRCC). (67) The kidney tumors in affected individuals are most often solitary and unilateral and have a mix of histologic patterns; they can be papillary, tubulopapillary or solid. The hallmark of hereditary leiomyomatosis renal cell carcinoma is the presence of a large nucleus with prominent orangophilic nucleoli. (68) (Figure 6) Germline mutations of the *FH* gene have been detected in over 90% of North American individuals with hereditary leiomyomatosis renal cell carcinoma. (69–71) Inhibition of *FH* leads to increased levels of fumarate that inhibit the activity of HIF prolyl hydroxylase, resulting in HIF- $\alpha$  accumulation and transcriptional upregulation of HIF-target genes, thereby contributing to the highly aggressive nature of HLRCC-associated renal tumors. (72) The inhibition of HIF prolyl hydroxylase by the increased levels of fumarate provides a VHL independent mechanism for dysregulation of HIF degradation in *FH*-deficient HLRCC-associated kidney cancers. (Figure 7A)

*FH*-deficient kidney cancer cell lines are glucose dependent(73;74) and have significantly impaired oxidative phosphorylation. (73;75) Sudarshan *et al.* (74) have demonstrated that the glucose-mediated generation of cellular reactive oxygen species in an *FH*-deficient kidney cancer cell line results in stabilization of HIF1- $\alpha$ . The impaired oxidative phosphorylation in *FH*-deficient kidney cancer results in a nearly total dependence on glycolysis for energy production. Downstream HIF1- $\alpha$  genes such as *VEGF* and *GLUT1* would therefore be critically important to these cancer cells for increasing vasculature and increasing glucose transport. Tumor vasculature and glucose transport could be the Achilles heel for these aggressive cancers that are so dependent on glucose transport for energy production. Whereas targeting the vasculature in patients with advanced VHL-deficient clear cell kidney cancer has resulted in only modest success, targeting the vasculature in a cancer whose metabolism is so completely dependent on glucose transport for energy production could provide a more powerful and effective approach to therapy.

### Succinate Dehydrogenase: Familial Renal Cancer

Germline mutations in three of the four *succinate dehydrogenase* genes (*SDHB*, *SDHC*, and *SDHD*) have been associated with familial paraganglioma and familial pheochromocytoma. (76;77) Individuals with these hereditary conditions are at risk for the development of extra-adrenal paraganglioma or pheochromocytoma and carotid body tumors. Recently early onset renal tumors have been found to develop in individuals with germline *SDHB* mutations. (77–81) In pre-clinical models increased succinate has been shown to inhibit HIF prolyl hydroxylase and affect HIF stability. (72) Inactivation of succinate dehydrogenase would be expected to severely impair oxidative phosphorylation and lead to glucose dependence of SDH-deficient kidney tumors. (Figure 7B) It is likely that SDH-deficient kidney tumors would be as sensitive to glucose as FH-deficient tumors and an approach to targeting the vasculature and/or glucose transport in these tumors could provide a novel approach to disrupt the fundamental metabolic machinery of these aggressive cancers.

### Discussion

Mutations in each of the seven known kidney cancer genes lead to the dysregulation of at least one metabolic pathway that is mediated by oxygen, iron, energy and/or nutrient sensing suggesting that kidney cancer is a disease of dysregulated cellular metabolism. The common endpoint resulting from *VHL*, *FH* and *SDH* mutations is the stabilization of HIF through inactivation of PDH, driving the transcriptional activation of genes that support tumor growth, neovascularization, invasion and metastasis. On the other hand HIF translation is upregulated by activation of the mTOR pathway as a consequence of its dysregulation by *TSC1/TSC2* or *FLCN* mutations, or oncogenic HGF/MET signaling through RAS-Erk1/2-Mek1/2-p90<sup>RSK</sup> phosphorylation of LKB1. Targeting HIF and its target genes has provided a first line therapeutic approach to VHL-deficient kidney tumors that may be applied to FH- and SDH-deficient tumors as well.

Progress has been made in this approach to targeted therapy for kidney cancer in recent years. Six novel agents focusing on the VHL pathway have been approved for the treatment of patients with advanced kidney cancer. However, even the most commonly used agent, sunitinib, has a partial response rate of only 31% and a progression free advantage over interferon of 6 months (11 versus 5 months). (82) Most patients with advanced kidney cancer treated with targeted agents eventually progress and there are very few long term complete responses. The need for novel therapeutic approaches for this tumor type is underscored by the eventual failure of the presently available therapeutic treatments. One report describing a novel agent, STF-62247, which targets autophagy selectively in VHL-deficient clear cell kidney cancer is an excellent example of an innovative approach to therapy for VHL-deficient tumors and highlights the role of metabolic defects in this cancer. (44)

It is possible we could spend the next decade performing clinical trials evaluating different combinations of tyrosine kinase inhibitors targeting a limited number of downstream HIF pathway genes with only minimal incremental improvement in outcome. Thinking about kidney cancer as a metabolic disease and targeting the kidney cancer gene metabolic abnormalities provides a potentially different paradigm for the development of therapy for this disease. As we learn more about the metabolic basis of kidney cancer and we start to think about these cancers in a different way, we may find that the importance of the oxidative phosphorylation status of an individual tumor may rival the activation status of downstream HIF targets in guiding rational targeted therapeutic approaches.

The two best examples of a metabolic basis for kidney cancer are fumarate hydratase and succinate dehydrogenase deficient kidney cancers, which represent the “Warburg effect” in cancer. (83) The FH-deficient UOK262 kidney cancer cell line, established from an HLRCC-

associated renal tumor, has been shown to have severely impaired oxidative phosphorylation and to be dependent on glycolysis for energy production. This is supported by ultrastructural analysis of UOK262 kidney cancer cells, which revealed abnormal mitochondria with significantly distorted cristae.(73) These FH-deficient tumor cells have significantly increased glucose transport and are extremely dependent on glucose for growth.(73) FH deficient HLRCC tumors in patients express high levels of the glucose transporter, GLUT1, and these tumors characteristically demonstrate high uptake of <sup>18</sup>F-FDG on PET scans.(72;73) Targeting the vasculature and impairing glucose transport in a glucose-addicted tumor could provide a new paradigm for treatment of these lethal malignancies. Interestingly, although the genetic basis of sporadic chromophobe renal carcinoma and oncocytomas is not known, ultrastructural analysis these renal neoplasms reveals abnormal mitochondria with altered cristae also suggestive of compromised mitochondrial function (84). The mitochondrial defects shared by FH-deficient tumors, sporadic chromophobe renal tumors and renal oncocytomas may suggest a common approach to therapy for these tumor types.

Treatment with inhibitors of mTOR activity has been applied to TSC2-deficient tumors and may show promise for FLCN-deficient tumors in the future. Furthermore, there has been renewed interest in agents such as metformin that activate AMPK, which in turn downregulates mTOR activity, as a potential therapeutic treatment for kidney cancer. Metformin use has been associated with a decreased cancer mortality in type II diabetics(85;86) and has been shown to block tumor growth in combination with chemotherapeutic agents in preclinical *in vitro* and *in vivo* models of cancer.(87;88) Although metformin is taken by patients with type II diabetics worldwide and is a relatively non-toxic agent, there are risks associated with its long term use. (85) However, if such agents were to be effective components of a targeted therapeutic approach, the benefit would seem to far outweigh the potential risk.

If we think about the factors known to increase the incidence of kidney cancer, it is not surprising that kidney cancer is fundamentally a metabolic disease; numerous studies have shown a significant association between body mass index, obesity and the development of kidney cancer.(89) We have learned much from our study of the metabolic basis for kidney cancer which should enable us to predict the pathways likely to be involved in histologic types of kidney cancer with a lower incidence leading to the design of more effective therapies. Although the genetic basis of more rare types of kidney cancer such as medullary renal carcinoma is not known, it is likely that the origin of medullary renal cell carcinoma is associated with hypoxia. Medullary renal carcinoma is an aggressive form of kidney cancer almost exclusively seen in patients who are affected with sickle cell disease or trait.(90) Sickle cell disease can be associated with a number of nephropathies, including microangiopathy. (91) It would not be surprising to find medullary renal cell carcinoma developing from a hypoxic area of renal parenchyma which may show elevated levels of HIF and may respond to targeted anti-HIF therapies.

## Conclusions

Kidney cancer is fundamentally a metabolic disease; each of the inherited kidney cancer syndromes caused by germline mutations in the kidney cancer genes identified to date represent disorders in oxygen, iron, nutrient and/or energy sensing (Figure 1). Identification of the genes for kidney cancer has provided the foundation for development of targeted approaches to therapy for patients with these disorders. While there has been considerable progress in the development of novel therapeutic approaches for patients with kidney cancer, there remains a significant opportunity for targeting the common metabolic defects in these cancer gene pathways.



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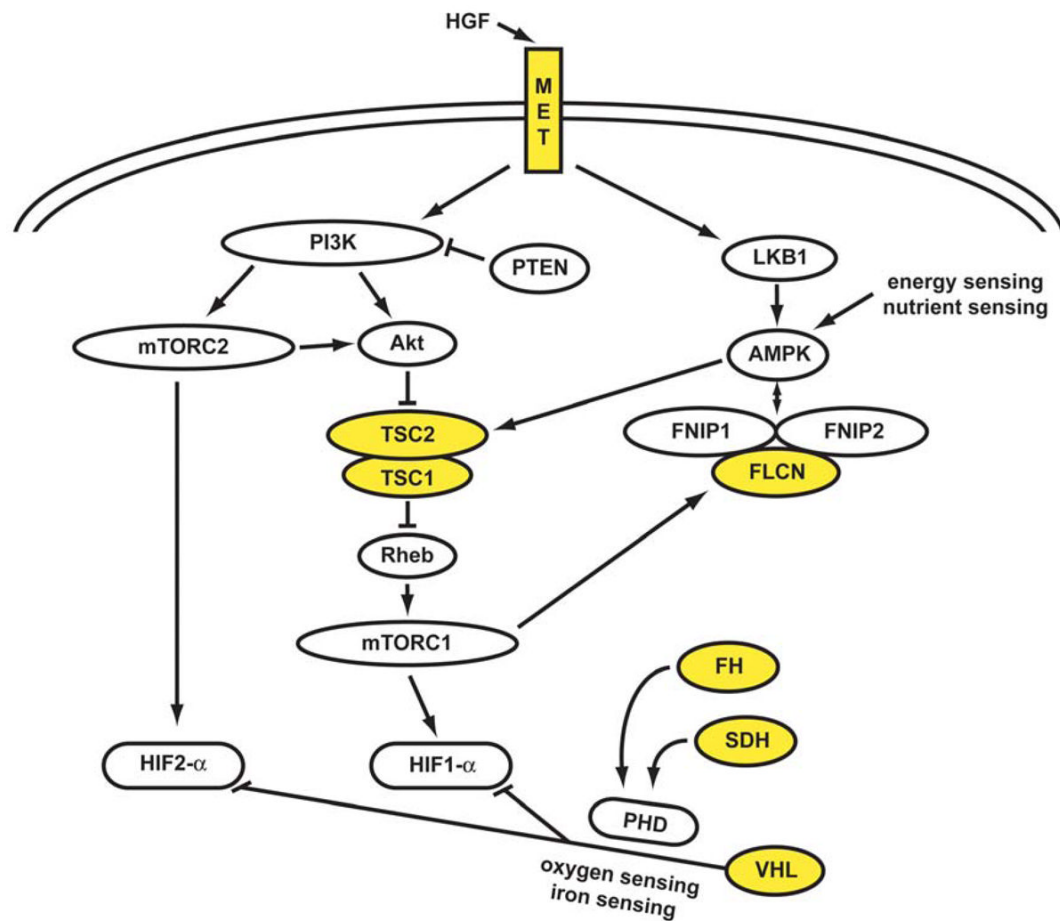
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## Biographies

W. Marston Linehan, M.D. is Chief of Urologic Oncology Branch at the National Cancer Institute, Bethesda, Maryland. For the last 28 years he has studied the genetic basis of cancer of the kidney. He and his colleagues have studied the *VHL* gene (von Hippel Lindau, clear cell renal carcinoma), the gene for Hereditary Papillary Renal Carcinoma (the *MET* proto-oncogene, type I papillary renal carcinoma), the *FLCN* gene (Birt Hogg Dubé syndrome, chromophobe renal carcinoma) and the germline fumarate hydratase mutations in the North American families with hereditary leiomyomatosis renal cell carcinoma (HLRCC). They work on the development of therapeutic strategies for the different types of kidney cancer based on understanding the molecular pathway of the specific cancer genes associated with the different types of kidney cancer.

Ramaprasad Srinivasan, M.D., Ph.D is a senior attending clinician in the Urologic Oncology Branch, National Cancer Institute. He obtained his M.B.B.S.degree in 1991 from Bangalore Medical College, India and went on to receive a Ph.D. in Biomedical Sciences from the University of Texas MD Anderson Cancer Center in 1996. He completed his residency training in Internal Medicine at the University of Texas Health Sciences Center, Houston and subsequently completed fellowship training in Medical Oncology and Hematology at the National Institutes of Health. He joined the Urologic Oncology Branch in 2003 and currently leads their targeted therapeutics program. His main research focus is the development of rational targeted therapeutic strategies in urologic malignancies.

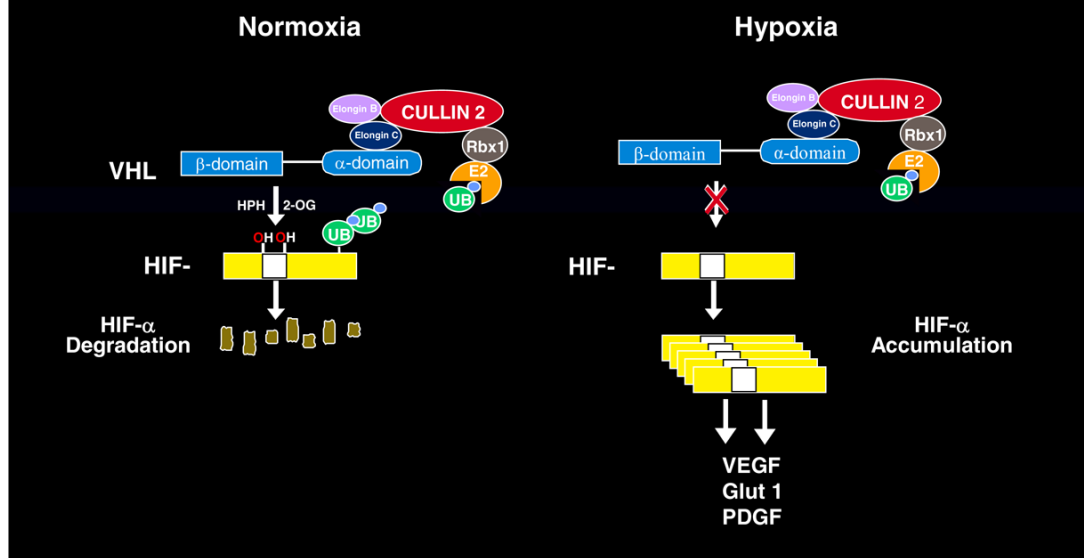
Laura S. Schmidt, Ph.D. received her Ph.D. degree in biochemistry from Vanderbilt University and was a Medical Foundation fellow at Tufts University School of Medicine in Boston. She currently holds the position of Principal Scientist in the Urologic Oncology Branch at the National Cancer Institute, National Institutes of Health. For the past 20 years her research interests have focused on the identification of new renal cancer predisposing genes through studies of families with inherited kidney cancer. Currently, her research is centered on understanding the function of the protein encoded by the *folliculin* gene and how germline mutations in *folliculin* increase risk for renal neoplasia in Birt-Hogg-Dube' syndrome.



### Figure 1. The genetic basis of kidney cancer: a metabolic disease

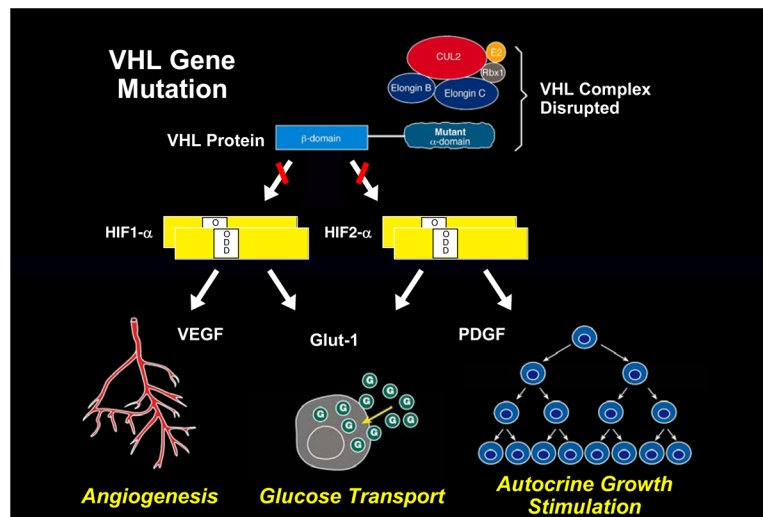
The genes known to cause kidney cancer, *VHL*, *MET*, *FLCN*, *FH*, *SDH*, *TSC1* and *TSC2* share the common feature that each is involved in oxygen, iron, energy and/or nutrient sensing pathways. Kidney cancer is fundamentally a metabolic disease. *VHL* targets HIF-1 $\alpha$  and HIF-2 $\alpha$  for ubiquitin-mediated degradation through an oxygen and iron sensing mechanism. The FLCN/FNIP1/FNIP2 complex binds AMPK, the primary energy sensor in the cell, and FLCN is phosphorylated by a rapamycin-sensitive kinase (i.e., mTORC1). TSC1/TSC2 are phosphorylated by the LKB1/AMPK cascade and help mediate the cell's response to energy/nutrient sensing. Fumarate hydratase and succinate dehydrogenase are TCA cycle enzymes. When fumarate hydratase or succinate dehydrogenase are deficient, the function of the TCA cycle is impaired and the cell is dependent on glycolysis for energy production. Inactivation of fumarate hydratase or succinate dehydrogenase impairs PHD function and represents a VHL-independent mechanism for dysregulation of HIF degradation. Increased HIF levels lead to increased GLUT1 which enables transport of glucose for ATP production. Abbreviations: folliculin interacting protein 1 (FNIP1) and folliculin interacting protein 2 (FNIP2), HIF prolyl hydroxylase (PHD). Adapted from Linehan, et al.(1)

## HIF $\alpha$ is targeted for degradation in normoxic, but not hypoxic cells



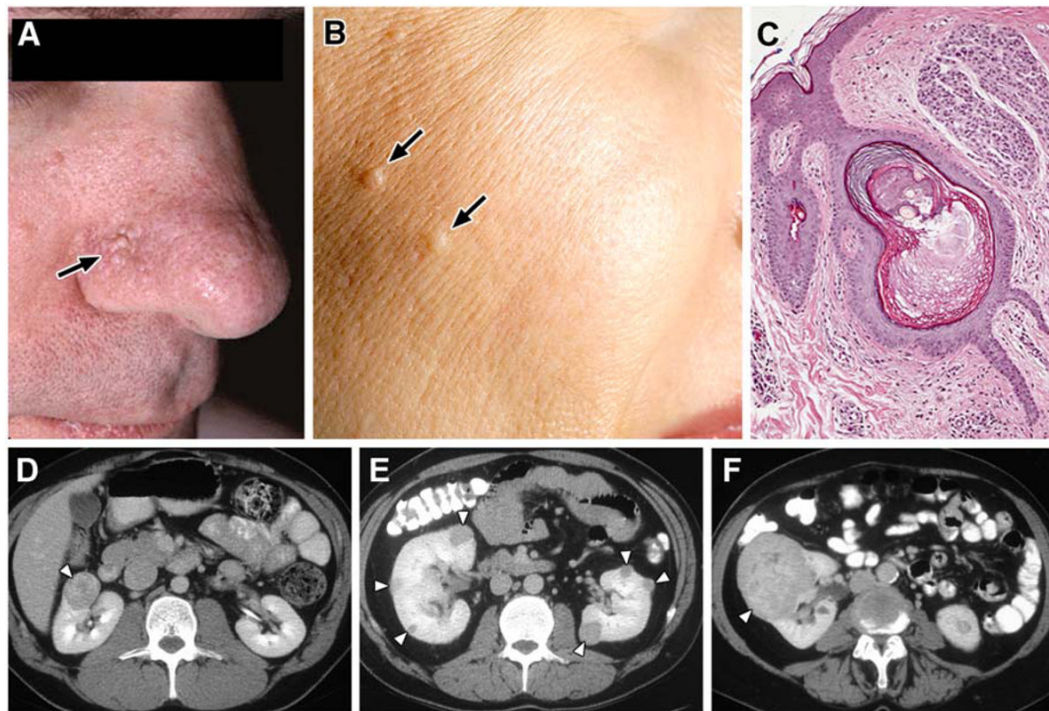
**Figure 2. The VHL complex targets HIF: an oxygen/iron sensing pathway**

The VHL E3 ubiquitin ligase complex targets the hypoxia inducible factors for ubiquitin-mediated degradation. This process is mediated by HIF prolyl hydroxylase (PHD) and an essential cofactor, 2-oxoglutarate (2-OG). This is an oxygen/iron sensing system; when the cell is hypoxic or iron levels are low, the VHL complex cannot target and degrade HIF and HIF over-accumulates, driving the transcription of a number of genes important in cancer, such as vascular endothelial growth factor (*VEGF*), platelet derived growth factor (*PDGF*) and the glucose transporter, *GLUT 1*.(2–4)



**Figure 3. The VHL complex targets HIF-1 $\alpha$  and HIF-2 $\alpha$  for degradation**

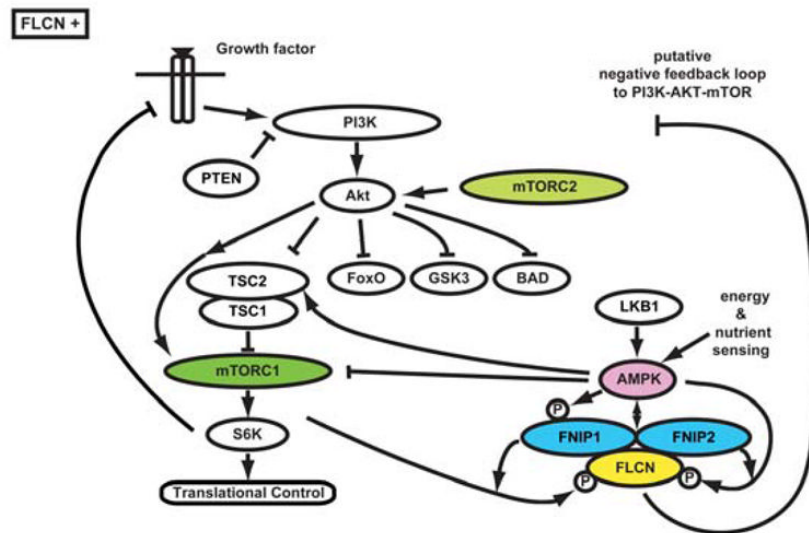
The VHL complex targets HIF-1 $\alpha$  and HIF-2 $\alpha$  for ubiquitin mediated degradation.(2) When the *VHL* gene is mutated, as in clear cell kidney cancer, the complex cannot target and degrade HIF. HIF-2 $\alpha$  is thought to be a critical pathway for VHL-deficient clear cell kidney tumorigenesis.(5;6)



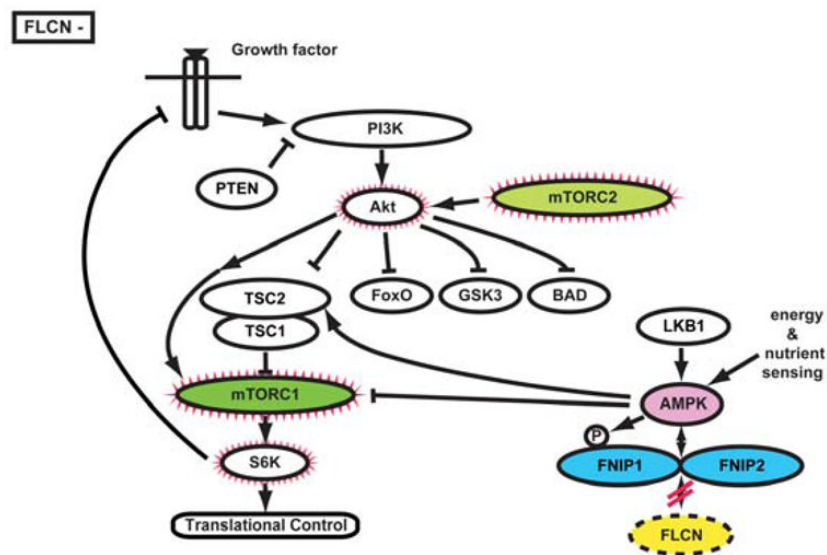
**Figure 4. Birt-Hogg-Dubé (BHD) syndrome: kidney cancer, cutaneous fibrofolliculomas and pulmonary cysts**

BHD patients are at risk for the development of benign cutaneous tumors (A, B) that may occur on the face and neck. These cutaneous lesions are benign fibrofolliculomas (C). BHD patients are at risk for the development of renal tumors that can be solitary (D), bilateral, multifocal (E) and can grow to large size and metastasize (F). From Pavlovich, et al.(7)





A

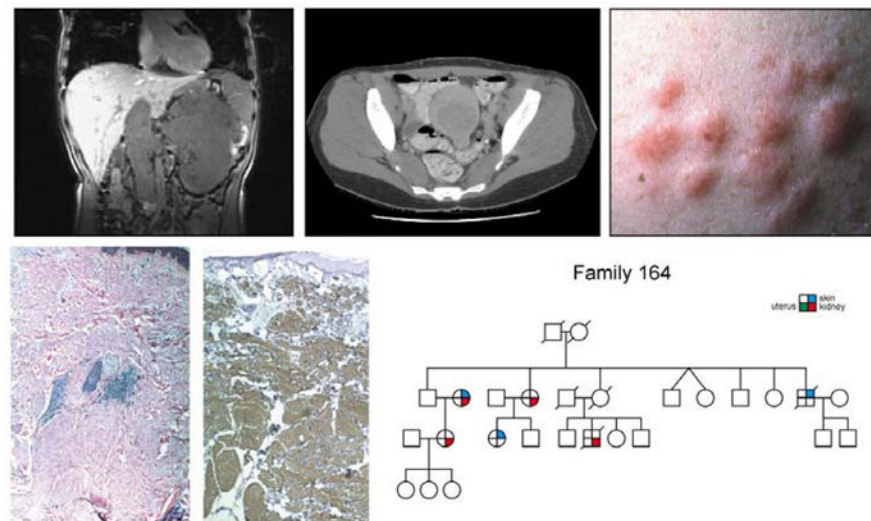


B

### Figure 5. The FLCN pathway

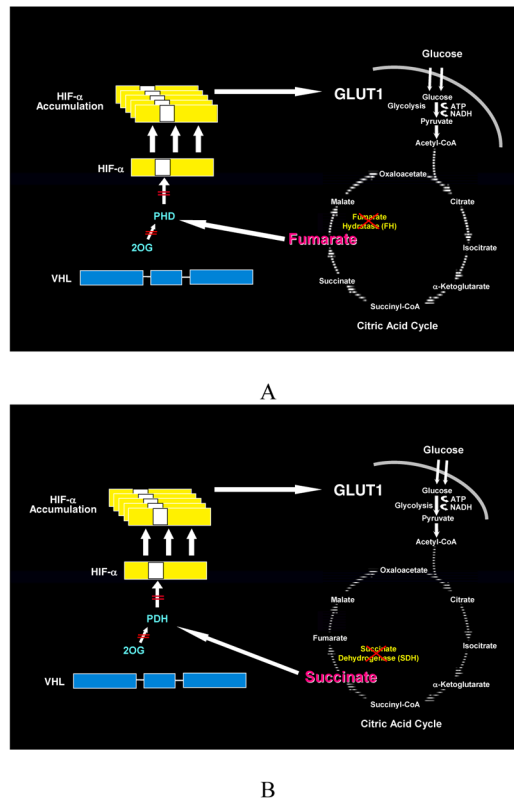
A. *FLCN* is the gene for the Birt-Hogg-Dubé (BHD) syndrome. Patients affected with BHD are characterized by germline mutation of the *FLCN* gene. The *FLCN*/FNIP1/FNIP2 complex binds AMPK and *FLCN* is phosphorylated by a rapamycin-sensitive kinase (i.e., mTORC1).

B. When *FLCN* is deficient, AKT, mTORC1 and mTORC2 are activated. From Hasumi, et al. (8)



**Figure 6. Hereditary leiomyomatosis renal cell cancer**

Hereditary leiomyomatosis renal cell cancer (HLRCC) is a hereditary cancer syndrome in which affected individuals are at risk for the development of kidney cancer (upper left panel) and uterine (upper middle panel) and cutaneous (upper right panel) leiomyomas (lower left and center panels). From Linehan et al.(9)



**Figure 7. Fumarate Hydratase and Succinate Dehydrogenase Deficient Kidney Cancer: the Warburg Effect**

Fumarate hydratase and succinate dehydrogenase-deficient kidney cancer are examples of the Warburg effect in cancer.(10) Germline fumarate hydratase mutations are found in the germline of patients affected with Hereditary Leiomyomatosis Renal Cell Cancer (HLRCC). HLRCC patients are at risk for the development of an aggressive form of kidney cancer. A) In fumarate hydratase deficient cells, fumarate accumulates and inhibits prolyl hydroxylation by competitively inhibiting HPH (A). HIF accumulates and increases the transcription of vascular endothelial growth factor and the glucose transporter, *GLUT-1*. Oxidative phosphorylation is impaired by the loss of the TCA cycle enzyme, fumarate hydratase, and the cells depend on glycolysis for energy production.(11;12) B) Succinate dehydrogenase is another TCA cycle enzyme which has also been associated with familial renal carcinoma. Increased levels of succinate have also been shown to inhibit HPH and a similar mechanism for tumorigenesis is likely for SDH-associated kidney cancer.(11)