

Identification of the Genes for Kidney Cancer: Opportunity for Disease-Specific Targeted Therapeutics

W. Marston Linehan,¹ Peter A. Pinto,¹ Ramaprasad Srinivasan,¹ Maria Merino,² Peter Choyke,³ Lynda Choyke,¹ Jonathan Coleman,¹ Jorge Toro,⁴ Gladys Glenn,⁴ Cathy Vocke,¹ Bert Zbar,¹ Laura S. Schmidt,^{1,5} Donald Bottaro,¹ and Len Neckers¹

Abstract Recent advances in understanding the kidney cancer gene pathways has provided the foundation for the development of targeted therapeutic approaches for patients with this disease. Kidney cancer is not a single disease; it includes a number of different types of renal cancers, each with different histologic features, a different clinical course, a different response to therapy, and different genes causing the defects. Most of what is known about the genetic basis of kidney cancer has been learned from study of the inherited forms of kidney cancer: von Hippel Lindau (*VHL* gene), hereditary papillary renal carcinoma (*c-Met* gene), Birt Hogg Dubé (*BHD* gene), and hereditary leiomyomatosis renal cell cancer (*fumarate hydratase* gene). These Mendelian single-gene syndromes provide a unique opportunity to evaluate the effectiveness of agents that target the VHL, c-Met, BHD, and fumarate hydratase pathways.

Kidney cancer affects 36,000 Americans annually, and nearly 12,000 die each year from this disease in the United States (1). Although patients with kidney cancer who present to their physician with localized disease often have long-term survival, patients who present with advanced disease have a 2-year survival of only 18% (2). Kidney cancer is not a single disease; it is made up of a number of different types of cancer that occur in the kidney (3), each with a different histologic type, a different clinical course, a different response to therapy, and different genes causing the defect (4, 5). Clear cell is the most common type (75%), papillary occurs in 10%, and chromophobe renal carcinoma occurs in 5%, with the remaining being made up of collecting duct, medullary, and oncocytoma. It is hoped that understanding the genetic basis of cancer of the kidney will lead to the development of effective forms of therapy for this disease (Fig. 1).

Authors' Affiliations: ¹Urologic Oncology Branch, ²Laboratory of Pathology, and ³Molecular Imaging Program, Center for Cancer Research and Genetic Epidemiology Branch; ⁴Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland; and ⁵Basic Research Program, Science Applications International Corporation-Frederick, Inc., National Cancer Institute-Frederick, Frederick, Maryland

Received 7/27/06; accepted 10/10/06.

Grant support: Intramural Research Program of the National Cancer Institute/NIH, Center for Cancer Research and federal funds from the National Cancer Institute/NIH under contract N01-CO-12400.

Presented at the Second Cambridge Conference on Innovations and Challenges in Renal Cancer, March 24-25, 2006, Cambridge, Massachusetts.

Note: The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. government.

Requests for reprints: W. Marston Linehan, Urologic Oncology Branch, National Cancer Institute, Room 1-5940, Building 10, Bethesda, MD 20892-1107. Phone: 301-496-6353; Fax: 301-402-0922; E-mail: wml@nih.gov.

©2007 American Association for Cancer Research.

doi:10.1158/1078-0432.CCR-06-1870

Hereditary Kidney Cancer

Kidney cancer occurs in both sporadic (noninherited) and hereditary (inherited) forms. Most of what is known about the genetic basis of kidney cancer has been learned from study of the hereditary forms of kidney cancer (6). There are four well-defined hereditary types of kidney cancer: von Hippel Lindau (VHL), hereditary papillary renal carcinoma (HPRC), Birt Hogg Dubé (BHD), and hereditary leiomyomatosis renal cell carcinoma (HLRCC).

VHL: Clear Cell Renal Carcinoma

VHL (OMIM 19330) is an inherited cancer syndrome in which affected individuals are at risk of the development of tumors in a number of organs, including the kidneys (7). Patients affected by VHL are at risk of the development of cerebellar and spinal hemangioblastomas, retinal angiomas, endolymphatic sac tumors (8), pancreatic neuroendocrine tumors (9), pheochromocytoma (10), and bilateral, multifocal kidney cancers (11). VHL-associated renal tumors are always clear cell renal carcinoma (12). It has been estimated that VHL patients are at risk of the development of up to 600 tumors and 1,100 cysts per kidney (13).

The VHL gene. Genetic linkage analysis was done in VHL kindreds to identify the *VHL* gene on the short arm of chromosome 3 (14). Germline mutations of the *VHL* gene are identified in nearly 100% of VHL families (15). Mutations of the *VHL* gene have also been found in a high percentage of tumors from patients with sporadic, noninherited clear cell renal carcinoma (16, 17). The *VHL* gene has the characteristics of a tumor suppressor gene, and alteration of both copies of the gene is found in VHL-associated tumors and sporadic clear cell renal carcinoma (Fig. 2).

Targeting the VHL pathway. The product of the *VHL* gene forms a complex with elongin C and elongin B (18, 19), CUL2

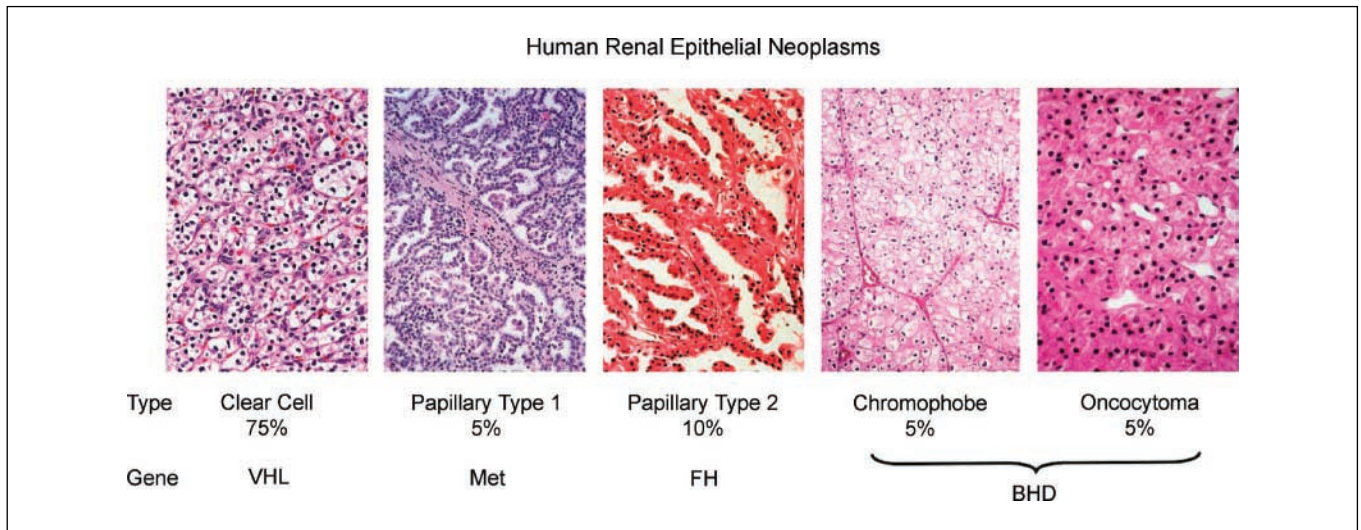


Fig. 1. Kidney cancer is not a single disease; it is made up of a number of different types of cancer that occur in the kidney, each with different histologic types, having a different clinical course, and associated with alteration of a different gene. From Linehan et al. (3).

(20), and RBX1 (21) to target the hypoxia-inducible factors HIF1 α and HIF2 α for ubiquitin-mediated degradation (22–24). Understanding the VHL pathway has provided the opportunity to develop therapies that target downstream HIF pathway genes *vascular endothelial growth factor* and *platelet-derived growth factor* with agents, such as sunitinib, that have high affinity for the vascular endothelial growth factor and platelet-derived growth factor receptors (25). Other approaches, such as targeting HIF transcription (26) and targeting HIF stability (27), are also being evaluated in clinical trials.

HPRC: Type 1 Papillary

HPRC (OMIM 164860) is a hereditary cancer syndrome, inherited in an autosomal dominant fashion, in which affected individuals are at risk of the development of bilateral, multifocal, type 1 papillary renal carcinoma (28, 29). It has been estimated that HPRC patients are at risk of the development of up to 3,000 tumors per kidney (30).

The HPRC gene: the MET gene. Genetic linkage analysis was done in HPRC kindreds, and the *MET* proto-oncogene was found to be the HPRC-causing gene (31). Activating mutations in the tyrosine kinase domain have been found in affected individuals in HPRC kindreds. HPRC is a highly penetrant hereditary cancer syndrome that tends to be late onset (32). Recently, however, an early-onset HPRC phenotype has been described (33). *MET* mutations have also been found in a subset of sporadic, type 1 papillary renal carcinomas (ref. 34; Fig. 3).

Oncogenic signaling via c-Met. The *MET* oncogene was isolated from a human osteogenic sarcoma cell line that had been chemically mutagenized *in vitro*. Transforming activity was due to a DNA rearrangement where sequences from the translocated promoter region (*TPR*) locus on chromosome 1 were fused to sequences from the *MET* locus on chromosome 7 (*TPR-MET*), a rearrangement that was later found in patients with gastric carcinoma (35, 36). Isolation of the full-length *MET* proto-oncogene coding sequence revealed structural

features of a membrane-spanning receptor tyrosine kinase (35). The identification of hepatocyte growth factor (HGF) as the natural ligand for the c-Met receptor protein and the identity of scatter factor (SF) and HGF united a collection of findings, showing that a single receptor transduced multiple biological activities, including motility, proliferation, survival, and morphogenesis (37–40).

The biochemical and biological effect of these *MET* mutants has been investigated in several model systems, confirming their suspected oncogenic potential (41–47). Trisomy of chromosome 7, which contains both *MET* and *HGF/SF* genes, occurs in 95% of sporadic papillary renal carcinoma (48); a detailed study of trisomy 7 in HPRC revealed nonrandom

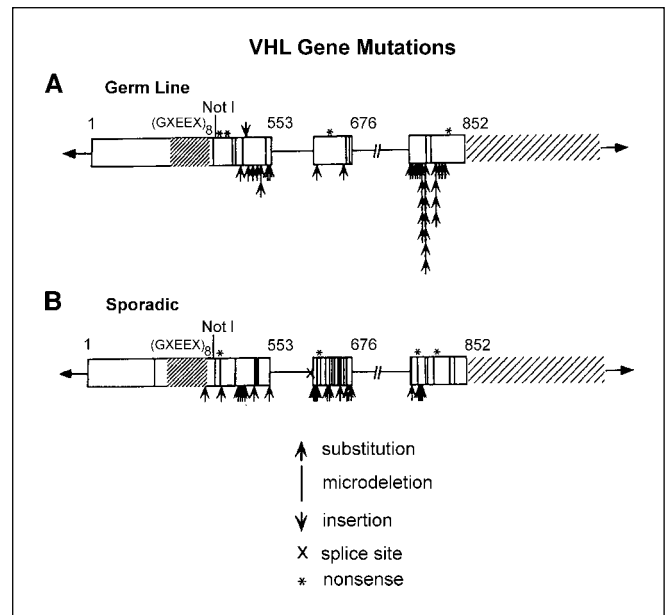
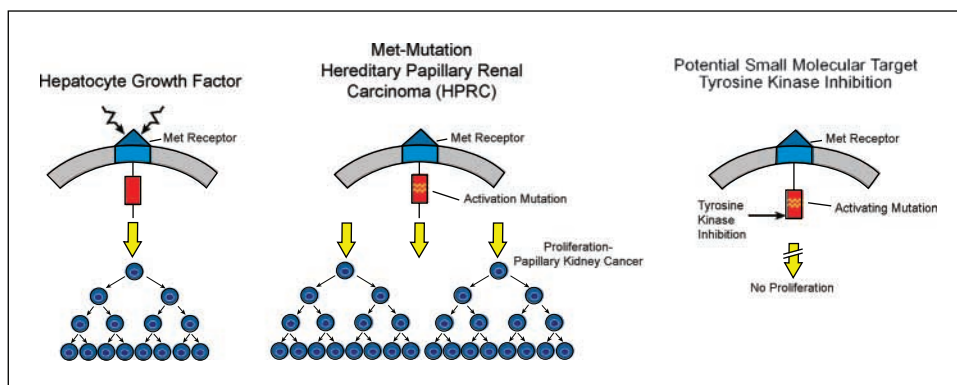


Fig. 2. Clear cell kidney cancer (A) is associated with mutation of the *VHL* gene (B). Adapted from Gnarr et al. (16).

Fig. 3. *MET* is the gene for HPRC. Activating mutations in the tyrosine kinase domain of the gene have been detected in the germ line of affected individuals in HPRC kindreds and in tumors from patients with sporadic type 1 papillary renal cell carcinoma. Intense efforts are under way to develop agents that block this pathway as a potential therapeutic approach for type 1 papillary renal cell carcinoma. From Linehan et al. (3).



duplication of the mutant *MET* allele in 100% of tumor samples (49).

Somatic *MET* mutations have since been found in several other human cancers, including gastric and liver cancer (50, 51), small cell and non-small cell lung cancers (52–54), and metastases of head and neck squamous cell carcinoma (55, 56). Unlike renal carcinoma, where mutations are typically confined to exons encoding the tyrosine kinase domain, these mutations encompass other receptor regions, most notably, the juxtamembrane region, where missense and deletion mutations that delay c-Met down-regulation occur with significant frequency (~12%) in lung cancers (54).

Cancer drug development targeting the c-Met pathway. At least three basic strategies have been used to target this pathway: antagonism of ligand/receptor interaction, inhibition of tyrosine kinase catalytic activity, and blockade of receptor/effector interactions. In addition, combinations of conventional and c-Met-targeted therapies may offer promise for specific cancers (57).

A collection of structure/function studies, including the early discovery that a naturally occurring truncated HGF/SF variant (HGF/NK2) was a specific competitive mitogenic antagonist, led to the development of HGF/NK4, a larger, more antagonistic HGF/SF fragment (58),⁶ and to an uncleavable form of pro-HGF/SF (59), both of which block tumor growth and metastasis in animal models. Similarly, the early development of c-Met ectodomain/IgG fusion protein with HGF/SF-neutralizing activity preceded the engineering of a soluble c-Met ectodomain fragments with pathway-neutralizing and antitumor activities (60, 61). Neutralizing mouse monoclonal antibodies against human HGF/SF have also been shown effective antitumor agents in animal models (62–64). The recent development of a fully human monoclonal antibody with HGF/SF-neutralizing and antitumor properties and its introduction into phase 1 human clinical trials are important steps forward (35, 65).⁷

Highly selective synthetic inhibitors of c-Met ATP binding, effective in the nanomolar concentrations in cultured cells, have been developed and tested in various model systems (66–73). Of these, the novel indolinone compounds SU11274 and PHA665752 displayed a minimum of 50-fold selectivity for c-Met relative to several other tyrosine kinases and potently

blocked HGF-stimulated activities in cultured cells and tumorigenicity in well-characterized c-Met-driven xenograft models (70). Analysis of SU11274 using cells that express HPRC-associated *MET* mutants revealed interesting differences in sensitivity (69), and gastric cancer cells with *MET* gene amplification displayed significantly increased sensitivity to PHA665752 (73), strongly reinforcing the concept that knowledge of genetic alterations should help predict the efficacy of c-Met tyrosine kinase inhibitors for specific patient groups. Not surprisingly, the number of pharmaceutical and biotechnology companies that have announced drug development programs targeting the c-Met tyrosine kinase has grown considerably in the last 3 years.⁸

The requirement of the COOH-terminal docking site for wild-type or mutant c-Met-transforming activity in cultured cells (43, 44) and the known roles of intracellular effectors, including Gab1, phosphatidylinositol 3-kinase, growth factor receptor binding protein 2, Src homology and collagen, and signal transducer and activator of transcription 3, in cell transformation (38, 40) suggest that targeting one or more of these interactions could effectively disrupt c-Met-driven oncogenesis. Knowledge of the unique structure of the growth factor receptor binding protein 2 SH2 domain provided the basis for the development of small synthetic growth factor receptor binding protein 2 selective binding antagonists (74). Further refinement of these early structures has yielded compounds that block HGF/SF-stimulated cell motility, matrix invasion, and morphogenesis in normal and tumor-derived cultured cells, as well as vascular endothelial cells, at low nanomolar concentrations (75).

Beyond effector targeting, compounds that block HSP90/client interactions, such as geldanamycin (76), also potently block c-Met oncogenic signaling (77), thus potently, in fact, as to suggest that other mechanisms of drug action may be involved (78). Phase 1 and 2 clinical trials of geldanamycin-related compounds are under way for a variety of cancers where the c-Met pathway is active. Combining agents, such as geldanamycin, which attenuate the supply of new receptors to the cell surface with inhibitors of other specific receptor functions, could lower the effective dose of each, reducing the likelihood of drug toxicity and the selection pressure for drug-resistant mutations.

⁶ <http://www.kringle-pharma.com/en/index.html>.

⁷ http://www.amgen.com/science/pipe_AMG102.html.

⁸ <http://www.exelixis.com/pipeline.shtml#XL880>, <http://www.amphoracorp.com/pipeline/default.aspx>, and <http://www.methylgene.com/content.asp>.

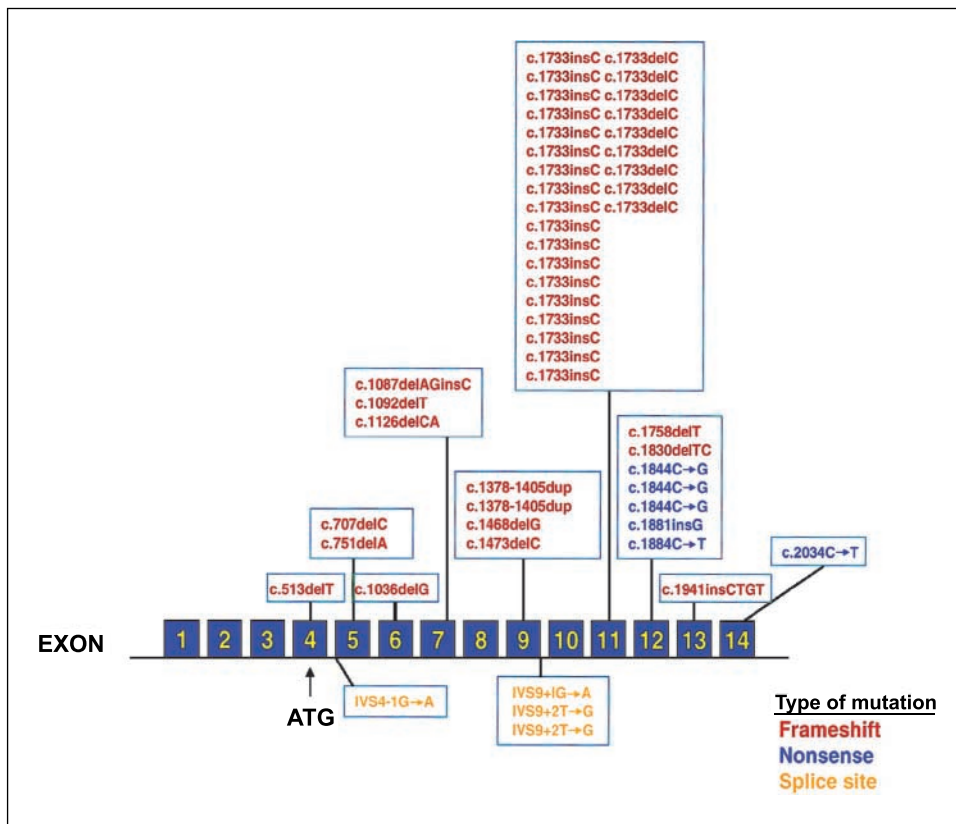


Fig. 4. Germline mutations of the *BHD* gene. Most germline mutations involve frameshift or nonsense mutations that are predicted to truncate the protein. From Schmidt et al. (85).

BHD: Chromophobe Renal Cell Carcinoma

BHD (OMIM 135150) is a hereditary cancer syndrome in which affected individuals are at risk of the development of cutaneous fibrofolliculomas (79), pulmonary cysts, and renal tumors (80, 81). BHD-associated renal tumors may be chromophobe renal cell carcinoma (33%), hybrid oncocyctic renal cell carcinoma (50%), clear cell renal cell carcinoma (9%), or oncocytoma (5%).⁹ Clinical management of BHD-associated renal tumors is similar to that of VHL and HPRC renal tumors; surgical removal is often recommended when the renal tumors reach the 3-cm threshold (82).

Identification of the *BHD* gene. Genetic linkage analysis was done in BHD kindreds to localize (83) the *BHD* gene on the short arm of chromosome 17 (84). Germline mutations of the *BHD* gene have been found in 51 (84%) of 61 BHD kindreds evaluated at the National Cancer Institute (85). Twenty-two different mutations have been identified, which are mainly frameshift or nonsense mutations (i.e., mutations that would be predicted to truncate the BHD protein folliculin). Mutations were distributed throughout the gene with no correlation between location or type of mutation and phenotypic features of BHD. More than half of BHD kindreds carry an insertion or deletion in a mononucleotide tract of eight cytosines within exon 11, representing a hypermutable "hotspot" for mutation in *BHD*. Patients who inherited the C deletion mutation developed renal tumors at a significantly lower frequency (6%)

than patients who inherited the C insertion mutation (33%; Fig. 4).

The *BHD* gene: a novel tumor suppressor gene. The *BHD* gene has the characteristics of a tumor suppressor gene. When Vocke et al. searched for somatic alterations of the *BHD* gene in tumor tissue from BHD patients carrying germline *BHD* mutations, somatic mutations or loss of heterozygosity was detected in 54 (70%) of 77 tumors; intragenic mutations were found in 41 of 77 tumors, and loss of heterozygosity of the 17p11.2 chromosomal region containing the *BHD* gene was found in 13 (17%) of 77 tumors (86). In all cases, the germline *BHD* mutant allele was retained, and the wild-type *BHD* allele was lost. Consistent with these results, *BHD* mRNA expression was hardly detectable in BHD-associated renal tumors by *in situ* hybridization (87). Studies are currently under way to identify the *BHD* gene function and to determine how alteration of this gene leads to kidney cancer (Fig. 5).

HLRCC

HLRCC (OMIM 605839) is a hereditary cancer syndrome in which affected individuals are at risk of the development of cutaneous and uterine leiomyomas and kidney cancer (88). HLRCC-associated kidney cancer is markedly different from that found in VHL, HPRC, or BHD. HLRCC-associated kidney cancer is an extremely aggressive form of the disease; it tends to spread early when the tumors are small. HLRCC kidney cancer is characterized by a microcystic histologic pattern with prominent organophilic nucleoli (89). Affected women in HLRCC kindreds are at risk of developing uterine leiomyomas.

⁹ <http://www.kosan.com/tech.productopps.htm>.

Nearly 90% of the affected women in the North American kindreds seen at the National Cancer Institute were found to have uterine leiomyoma, and 50% had had a hysterectomy before the age of 30 years (refs. 90, 91; Fig. 6).

The HLRCC gene: fumarate hydratase. The HLRCC gene was found to be the Krebs cycle enzyme *fumarate hydratase* (FH; ref. 92). Mutations of the FH gene have been found in 52 (93%) of 56 HLRCC kindreds evaluated at the National Cancer Institute. Thirty-one different germline FH mutations have been identified, consisting of 20 missense, 8 frame shifts (3 insertions and 5 deletions), 2 nonsense, and 1 splice site. Mutations were found throughout the gene except exon 5. Twenty (65%) of the 31 mutations resulted in the substitutions of single amino acid residues that were highly conserved (refs. 90, 91; Fig. 7).

FH Pathway. Recently, we reported that HLRCC tumor specimens expressed high levels of both HIF-1 α and HIF-2 α as well as the HIF target gene *GLUT1* when compared with adjacent renal epithelium (93). Elevated HIF levels could be traced to reduced HIF proline-hydroxylation, not to loss of VHL. Using enzyme kinetic analysis, we found fumarate to be a very potent competitive inhibitor of HIF prolyl hydroxylase. Furthermore, we showed that a small elevation in intracellular fumarate, whether induced pharmacologically or by molecular knockdown of FH, is sufficient to up-regulate HIF, GLUT1 expression, glucose uptake, and lactic acid production (Fig. 8). These findings identify biallelic loss of FH as a single gene defect capable of inducing the Warburg effect (the tendency of cancer cells to rely on glycolysis as their energy source). Further experimentation will be needed to fully explore the link between dysregulation of the tricarboxylic acid cycle and tumorigenesis and to more thoroughly elucidate the role of HIF in this process (Fig. 8).

Targeting the FH pathway in HLRCC kidney cancer. One approach currently being evaluated in HLRCC kidney cancer is to target the HIF pathway with agents such as bevacizumab. Another approach being evaluated is to target the HSP90 pathway with agents such as 17-allylamino-17-desmethoxygeldanamycin (17-AAG). 17AAG, a small-molecule inhibitor of Hsp90, the benzoquinone ansamycin, has shown antitumor activity in several human xenograft models and is currently in clinical trials, both as a single agent and in combination with

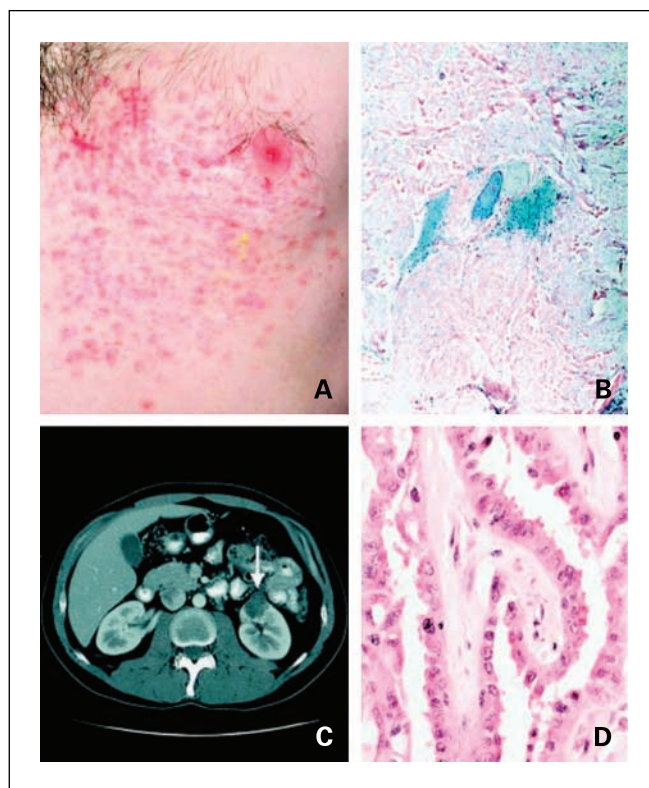


Fig. 6. HLRCC clinical, radiologic, and histologic manifestations can include cutaneous leiomyoma (A and B), uterine leiomyoma (data not shown), and kidney cancer (C and D). From Toro et al. (98).

other therapeutics. Recently, Vanharanta et al. (94), in an expression profile study, reported that NAD(P)H dehydrogenase quinone 1 (NQO1) was markedly overexpressed in FH-deficient uterine fibroids compared with wild-type FH-expressing fibroids. We confirmed very high expression of NQO1 protein in FH-deficient HLRCC tumor specimens compared with tumor tissue from other hereditary renal cancers (Fig. 9). High NQO1 expression predicts for enhanced sensitivity to 17-AAG. In one study, ectopic expression of NQO1 in NQO1-null colon carcinoma cells resulted in a 32-fold increase in the cytotoxicity of 17-AAG (95). This dramatic sensitization to 17-AAG is due to the ability of NQO1 to reduce the benzoquinone moiety of the drug, which results in its markedly improved antitumor activity (96). Thus, HLRCC is expected to be particularly sensitive to 17-AAG. Animal xenograft studies in support of this hypothesis are currently under way.

Conclusion

In summary, recent understanding of the kidney cancer gene pathways has provided the opportunity to develop pathway-specific therapies for the different histologic types of this disease. The potential opportunity in studying hereditary cancer syndromes was recently highlighted by Fishman and Porter, who attributed the difficulty of drug discovery to the paucity of targets solidly linked to major diseases. They propose as a solution to validate targets identified in rare Mendelian disorders, where the inheritance of a single-gene mutation is linked to the disease (97). They note that the relationship

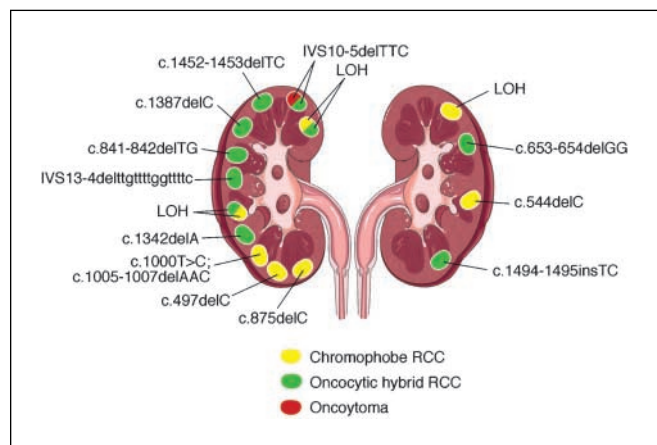


Fig. 5. The *BHD* gene has the characteristics of a tumor suppressor gene. Mutation or loss of the somatic allele of the *BHD* gene was detected in 70% of BHD-associated kidney tumors. From Vocke et al. (86).

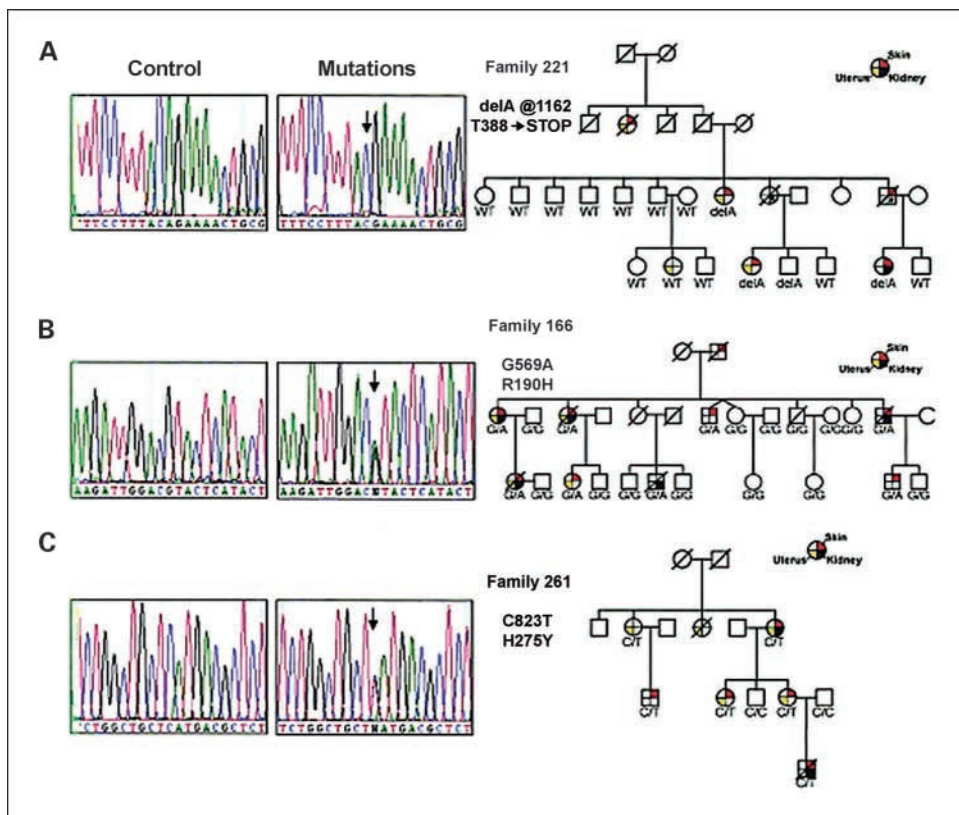


Fig. 7. Germline *FH* mutations in HLRCC kindreds. From Toro et al. (98).

between disease and the signaling pathway is clearest in genetic disorders, and that there is a clear rationale for extrapolating from genetic to sporadic disease (97).

Encouraging progress has been made with agents that target the VHL/HIF pathway in patients with advanced clear cell renal carcinoma, and clinical trials currently under way will provide insight into the clinical effectiveness of the combination of agents that target multiple parts of this pathway. Increased insights into the VHL, c-Met, BHD, and FH pathways should provide additional opportunities for the development of more effective forms of therapy for patients with each histologic type of kidney cancer.

Open Discussion

Dr. Atkins: How do you decide when to start treating patients with von Hippel-Lindau (VHL) with sunitinib?

Dr. Linehan: We have managed many people with many different kinds of tumors, and some of these people we have operated on two or three times. If you let the tumors keep growing, the patients will need surgery. If you let the tumors grow too big, they will spread.

Dr. Flaherty: We've considered using sorafenib in this patient population, but we have been cautious about using these drugs because of long-term tolerability. We've considered

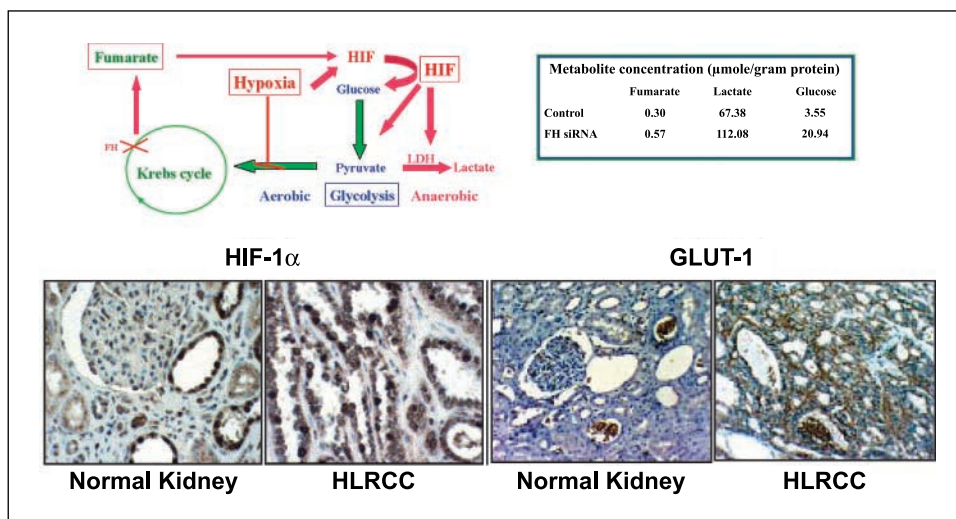
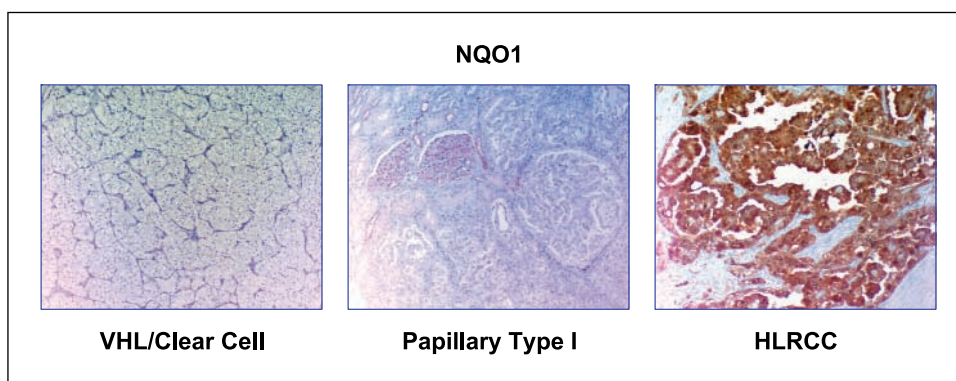


Fig. 8. Loss of FH impairs function of the tricarboxylic acid cycle and results in elevation of fumarate, which in turn stabilizes HIF. Elevated HIF drives transcription of key components of the glycolytic pathway, including GLUT1 and lactate dehydrogenase (*LDH*). Data in the table support the hypothesis that FH expression is inhibited by small interfering RNA (*siRNA*), as do HIF-1 α and GLUT1 immunocytochemistry of HLRCC tumor specimens compared with normal kidney tissue. Data were taken from Isaacs et al. (93).

Fig. 9. Markedly elevated NQO1 expression is restricted to HLRCC when compared with VHL/clear cell and type 1 papillary hereditary kidney cancer. NQO1 protein expression is visualized by immunocytochemistry.



starting at half-dose sorafenib, 200 mg twice a day, which had activity in phase 1 trials. It might not be a full drug test, but in patients in whom long-term therapy might be needed, more intensive therapy is probably not doable.

Dr. Linehan: When would you stop therapy?

Dr. Flaherty: With sorafenib at a continuous standard dose of 400 mg twice a day, patients tolerate the drug better after several months of continued therapy. At 200 mg twice a day, we figured it would be even more tolerable and less challenging. We have had patients with renal cell carcinoma and melanoma who have been taking sorafenib for a couple of years, and their tolerance of single-agent therapy is good.

Dr. Sosman: Dr. Linehan, could you go back to an earlier phase of the disease to determine when the patients have a certain number of renal tumors or renal cysts and do a randomized study?

Dr. Linehan: Do you think sunitinib or sorafenib would be a better agent to study?

Dr. Sosman: In terms of tolerability, I might say bevacizumab, but that is risky.

Dr. George: We studied PTK/ZK in 11 patients with VHL. We looked for objective response in patients with hemangioblastoma in the cerebellum and spine, which we didn't see. In addition, we used magnetic resonance imaging to determine different biologic effects. We are still analyzing that data. In terms of prevention, we have little experience with the long-term use of these drugs. It is difficult to determine therapy length and risks. We have seen complications, including spontaneous hemorrhage and hemangioblastoma.

Dr. McDermott: I have two patients with VHL who are currently receiving sorafenib. The first patient is a 56-year-old woman who has had metastatic kidney cancer for 7 years. She initially received interleukin 2 and achieved a PR. However, over the last 2 years, she has developed slow disease progression at several sites. She wondered if she should take sorafenib. Because of bleeding risk, I was hesitant to prescribe

it because she did not have metastatic disease symptoms. Earlier this year, she developed leg weakness, secondary to renal cell carcinoma metastasis to her spine. After recovering from spinal surgery, she began taking sorafenib and has tolerated it without complication for 3 months. The second patient is a 44-year-old man with all of the known complications of VHL syndrome. He was referred to me to receive sorafenib for his metastatic kidney cancer to the brain and skin. His skin lesions have resolved, and his brain tumor has gotten smaller. However, because he was debilitated from his VHL, he could not tolerate continuous dosing of sorafenib. Furthermore, he has experienced gross hematuria, likely from a retroperitoneal mass that has eroded into his ureter. While my experience is limited, because this class of drugs will not cure kidney cancer in patients with VHL, I would only recommend using them in patients with symptomatic kidney cancer because of the potential complications in both the malignant and benign tumors. Carefully monitored clinical trials for patients with VHL and renal cell carcinoma, while difficult to complete, should be initiated.

Dr. Atkins: Are there sporadic variants of hereditary leiomyomatosis renal cancer (HLRCC), and are there abnormalities in Birt Hogg Dubé (*BHD*) gene in patients with chromophobe tumors?

Dr. Linehan: For the HLRCC, if they exist, they are unusual. The BHD mutations have not been looked at extensively; however, in early attempts they have been found only infrequently.

Dr. Libermann: Do you see any deregulations of the fumarate hydratase enzyme in sporadic clear cell renal cancer?

Dr. Linehan: We have not looked at clear cell tumors for alterations of fumarate hydratase. The model that we proposed suggests that overaccumulation of fumarate essentially inactivates prolyl hydroxylases and then results in overaccumulation of HIF. This mechanism, however, may be only a small part of what we are dealing with in fumarate hydratase mutations.

References

1. Jemal A, Murray T, Ward E, et al. Cancer statistics, 2005. *CA Cancer J Clin* 2005;55:10–30.
2. Linehan WM, Yang JC, Bates SE. Cancer of the kidney. In: DeVita SE, Hellman S, Rosenberg SA, editors. *Cancer principles & practice of oncology*. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 1139–68.
3. Linehan WM, Walther MM, Zbar B. The genetic basis of cancer of the kidney. *J Urol* 2003;170: 2163–72.
4. Linehan WM, Zbar B. Focus on kidney cancer. *Cancer Cell* 2004;6:223–8.
5. Linehan WM, Vasselli J, Srinivasan R, et al. Genetic basis of cancer of the kidney: disease-specific approaches to therapy. *Clin Cancer Res* 2004;10: 6282–9S.
6. Zbar B, Klausner R, Linehan WM. Studying cancer families to identify kidney cancer genes. *Annu Rev Med* 2003;54:217–33.
7. Lonsler R, Glenn G, Walther MM. von Hippel-Lindau disease. *Lancet* 2003;361:2059–67.

8. Choo D, Shotland L, Mastroianni M, et al. Endolymphatic sac tumors in von Hippel-Lindau disease. *J Neurosurg* 2004;100:480–7.
9. Lubensky IA, Pack S, Ault D, et al. Multiple neuroendocrine tumors of the pancreas in VHL patients: histopathological and molecular genetic analysis. *Am J Pathol* 1998;153:223–31.
10. Walther MM, Keiser HR, Linehan WM, Choyke P, Rayford W, Lyne JC. Management of hereditary pheochromocytoma in von Hippel Lindau kindreds with partial adrenalectomy. *J Urol* 1999;161:395–8.
11. Grubb RL, Choyke P, Pinto PA, Linehan WM, Walther MM. Management of von Hippel-Lindau-associated kidney cancer. *Nature Clin Pract Urol* 2005;2:248–55.
12. Poston CD, Jaffe GS, Lubensky IA, et al. Characterization of the renal pathology of a familial form of renal cell carcinoma associated with von Hippel-Lindau disease: clinical and molecular genetic implications. *J Urol* 1995;153:22–6.
13. Walther MM, Lubensky IA, Venzon D, Zbar B, Linehan WM. Prevalence of microscopic lesions in grossly normal renal parenchyma from patients with von Hippel-Lindau disease, sporadic renal cell carcinoma and no renal disease: clinical implications. *J Urol* 1995;154:2010–5.
14. Latif F, Tory K, Gnarr JR, et al. Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science* 1993;260:1317–20.
15. Stolle CA, Glenn G, Zbar B, et al. Improved detection of germline mutations in the von Hippel-Lindau disease tumor suppressor gene. *Hum Mutat* 1998;12:417–23.
16. Gnarr JR, Tory K, Weng Y, et al. Mutation of the VHL tumor suppressor gene in renal carcinoma. *Nat Genet* 1994;7:85–90.
17. Shuin T, Kondo K, Torigoe S, et al. Frequent somatic mutations and loss of heterozygosity of the von Hippel-Lindau tumor suppressor gene in primary human renal cell carcinomas. *Cancer Res* 1994;54:2852–5.
18. Duan DR, Pause A, Burgess WH, et al. Inhibition of transcription elongation by the VHL tumor suppressor protein. *Science* 1995;269:1402–6.
19. Kibel A, Iliopoulos O, DeCaprio JA, Kaelin WG, Jr. Binding of the von Hippel-Lindau tumor suppressor protein to Elongin B and C. *Science* 1995;269:1444–6.
20. Pause A, Lee S, Worrell RA, et al. The von Hippel-Lindau tumor-suppressor gene product forms a stable complex with human CUL-2, a member of the Cdc53 family of proteins. *Proc Natl Acad Sci U S A* 1997;94:2156–61.
21. Kamura T, Koepf DM, Conrad MN, et al. Rbx1, a component of the VHL tumor suppressor complex and SCF ubiquitin ligase. *Science* 1999;284:657–61.
22. Iliopoulos O, Jiang C, Levy AP, Kaelin WG, Goldberg MA. Negative regulation of hypoxia-inducible genes by the von Hippel-Lindau protein. *Proc Natl Acad Sci U S A* 1996;93:10595–9.
23. Kaelin WG, Jr, and Maher ER. The VHL tumour-suppressor gene paradigm. *Trends Genet* 1998;14:423–6.
24. Kaelin WG, Jr. The von Hippel-Lindau gene, kidney cancer, and oxygen sensing. *J Am Soc Nephrol* 2003;14:2703–11.
25. Motzer RJ, Michaelson MD, Redman BG, et al. Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. *J Clin Oncol* 2006;24:16–24.
26. Rapisarda A, Uranchimeg B, Sordet O, Pommier Y, Shoemaker RH, Mellillo G. Topoisomerase I-mediated inhibition of hypoxia-inducible factor 1: mechanism and therapeutic implications. *Cancer Res* 2004;64:1475–82.
27. Isaacs JS, Jung YJ, Mimnaugh EG, Martinez A, Cuttitta F, Neckers LM. Hsp90 regulates a von Hippel-Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway. *J Biol Chem* 2002;277:29936–44.
28. Zbar B, Tory K, Merino M, et al. Hereditary papillary renal cell carcinoma. *J Urol* 1994;151:561–6.
29. Lubensky IA, Schmidt L, Zhuang Z, et al. Hereditary and sporadic papillary renal carcinomas with c-met mutations share a distinct morphological phenotype. *Am J Pathol* 1999;155:517–26.
30. Ornstein DK, Lubensky IA, Venzon D, Zbar B, Linehan WM, Walther MM. Prevalence of microscopic tumors in normal appearing renal parenchyma from patients with hereditary papillary renal cancer. *J Urol* 2000;163:431–3.
31. Schmidt L, Duh F-M, Chen F, et al. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. *Nat Genet* 1997;16:68–73.
32. Schmidt L, Junker K, Weirich G, et al. Two North American families with hereditary papillary renal carcinoma and identical novel mutations in the MET proto-oncogene. *Cancer Res* 1998;58:1719–22.
33. Schmidt LS, Nickerson ML, Angeloni D, et al. Early onset hereditary papillary renal carcinoma: germline missense mutations in the tyrosine kinase domain of the Met proto-oncogene. *J Urol* 2004;172:1256–61.
34. Schmidt L, Junker K, Kinjerski T, et al. Novel mutations of the MET proto-oncogene in papillary renal carcinomas. *Oncogene* 1999;18:2343–50.
35. Furge KA, Zhang YW, VandeWoude GF. Met receptor tyrosine kinase: enhanced signaling through adapter proteins. *Oncogene* 2000;19:5582–9.
36. Yu J, Miehlike S, Ebert MP, et al. Frequency of TPR-MET rearrangement in patients with gastric carcinoma and in first-degree relatives. *Cancer* 2000;88:1801–6.
37. Birchmeier C, Birchmeier W, Gherardi E, VandeWoude GF. Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 2003;4:915–25.
38. Zhang YW, VandeWoude GF. HGF/SF-met signaling in the control of branching morphogenesis and invasion. *J Cell Biochem* 2003;88:408–17.
39. Rosario M, Birchmeier W. How to make tubes: signaling by the Met receptor tyrosine kinase. *Trends Cell Biol* 2003;13:328–35.
40. Corso S, Comoglio PM, Giordano S. Cancer therapy: can the challenge be MET? *Trends Mol Med* 2005;11:284–92.
41. Jeffers M, Schmidt L, Nakaigawa N, et al. Activating mutations for the met tyrosine kinase receptor in human cancer. *Proc Natl Acad Sci U S A* 1997;94:11445–50.
42. Jeffers M, Fiscella M, Webb CP, Anver M, Koochekpour S, VandeWoude GF. The mutationally activated Met receptor mediates motility and metastasis. *Proc Natl Acad Sci U S A* 1998;95:14417–22.
43. Bardelli A, Longati P, Gramaglia D, et al. Uncoupling signal transducers from oncogenic MET mutants abrogates cell transformation and inhibits invasive growth. *Proc Natl Acad Sci U S A* 1998;95:14379–83.
44. Giordano S, Maffe A, Williams TA, et al. Different point mutations in the met oncogene elicit distinct biological properties. *FASEB J* 2000;14:399–406.
45. Michieli P, Basilico C, Pennacchietti S, et al. Mutant Met-mediated transformation is ligand-dependent and can be inhibited by HGF antagonists. *Oncogene* 1999;18:5221–31.
46. Miller M, Ginalski K, Lesyng B, Nakaigawa N, Schmidt L, Zbar B. Structural basis of oncogenic activation caused by point mutations in the kinase domain of the MET proto-oncogene: modeling studies. *Proteins* 2002;44:32–43.
47. Chiara F, Michieli P, Pugliese L, Comoglio PM. Mutations in the met oncogene unveil a "dual switch" mechanism controlling tyrosine kinase activity. *J Biol Chem* 2003;278:29352–8.
48. Kovacs G. Molecular cytogenetics of renal cell tumors. *Adv Cancer Res* 1993;62:89–124.
49. Zhuang Z, Park WS, Pack S, et al. Trisomy 7-harboring non-random duplication of the mutant MET allele in hereditary papillary renal carcinomas. *Nat Genet* 1998;20:66–9.
50. Lee JH, Han SU, Cho H, et al. A novel germ line juxtamembrane Met mutation in human gastric cancer. *Oncogene* 2000;19:4947–53.
51. Park WS, Dong SM, Kim SY, et al. Somatic mutations in the kinase domain of the Met/hepatocyte growth factor receptor gene in childhood hepatocellular carcinomas. *Cancer Res* 1999;59:307–10.
52. Maulik G, Kijima T, Ma PC, et al. Modulation of the c-Met/hepatocyte growth factor pathway in small cell lung cancer. *Clin Cancer Res* 2002;8:620–7.
53. Ma PC, Kijima T, Maulik G, et al. c-MET mutational analysis in small cell lung cancer: novel juxtamembrane domain mutations regulating cytoskeletal functions. *Cancer Res* 2003;63:6272–81.
54. Kong-Beltran M, Seshagiri S, Zha J, et al. Somatic mutations lead to an oncogenic deletion of Met in lung cancer. *Cancer Res* 2006;66:283–9.
55. Di Renzo MF, Olivero M, Martone T, et al. Somatic mutations of the MET oncogene are selected during metastatic spread of human HNSC carcinomas. *Oncogene* 2000;19:1547–55.
56. Lorenzato A, Olivero M, Patane S, et al. Novel somatic mutations of the MET oncogene in human carcinoma metastases activating cell motility and invasion. *Cancer Res* 2002;62:7025–30.
57. Aebbersold DM, Landt O, Berthou S, et al. Prevalence and clinical impact of Met Y1253D-activating point mutation in radiotherapy-treated squamous cell cancer of the oropharynx. *Oncogene* 2003;22:8519–23.
58. Matsumoto K, Nakamura T. Mechanisms and significance of bifunctional NK4 in cancer treatment. *Biochem Biophys Res Commun* 2005;333:316–27.
59. Mazzone M, Basilico C, Cavassa S, et al. An uncleavable form of pro-scatter factor suppresses tumor growth and dissemination in mice. *J Clin Invest* 2004;114:1418–32.
60. Michieli P, Mazzone M, Basilico C, et al. Targeting the tumor and its microenvironment by a dual-function decoy Met receptor. *Cancer Cell* 2004;6:61–73.
61. Kong-Beltran M, Stamos J, Wickramasinghe D. The Sema domain of Met is necessary for receptor dimerization and activation. *Cancer Cell* 2004;6:75–84.
62. Cao B, Su Y, Oskarsson M, et al. Neutralizing monoclonal antibodies to hepatocyte growth factor/scatter factor (HGF/SF) display antitumor activity in animal models. *Proc Natl Acad Sci U S A* 2001;98:7443–8.
63. Martens T, Schmidt N-O, Eckerich C, et al. Inhibition of intracerebral glioblastoma growth by treatment with a novel one-armed anti-Met antibody [abstract]. *GMS/German Med Sci*, an e-journal 2005.
64. Kim KJ, Wang L, Su YC, et al. Systemic anti-hepatocyte growth factor monoclonal antibody therapy induces the regression of intracranial glioma xenografts. *Clin Cancer Res* 2006;12:1292–8.
65. Burgess T, Coxon A, Meyer S, et al. Fully human monoclonal antibodies to hepatocyte growth factor with therapeutic potential against hepatocyte growth factor/c-Met-dependent human tumors. *Cancer Res* 2006;66:1721–9.
66. Christensen JG, Schreck R, Burrows J, et al. A selective small molecule inhibitor of c-Met kinase inhibits c-Met-dependent phenotypes *in vitro* and exhibits cytoreductive antitumor activity *in vivo*. *Cancer Res* 2003;63:7345–55.
67. Wang X, Le P, Liang C, et al. Potent and selective inhibitors of the Met [hepatocyte growth factor/scatter factor (HGF/SF) receptor] tyrosine kinase block HGF/SF-induced tumor cell growth and invasion. *Mol Cancer Ther* 2003;2:1085–92.
68. Sattler M, Pride YB, Ma P, et al. A novel small molecule met inhibitor induces apoptosis in cells transformed by the oncogenic TPR-MET tyrosine kinase. *Cancer Res* 2003;63:5462–9.
69. Berthou S, Aebbersold DM, Schmidt LS, et al. The Met kinase inhibitor SU11274 exhibits a selective inhibition pattern toward different receptor mutated variants. *Oncogene* 2004;23:5387–93.
70. Christensen JG, Burrows J, Salgia R. c-Met as a target for human cancer and characterization of inhibitors for therapeutic intervention. *Cancer Lett* 2005;225:1–26.

71. Ma PC, Schaefer E, Christensen JG, Salgia R. A selective small molecule c-MET inhibitor, PHA665752, cooperates with rapamycin. *Clin Cancer Res* 2005;11:2312–9.
72. Ma PC, Jagadeeswaran R, Jagadeesh S, et al. Functional expression and mutations of c-Met and its therapeutic inhibition with SU11274 and small interfering RNA in non-small cell lung cancer. *Cancer Res* 2005;65:1479–88.
73. Smolen GA, Sordella R, Muir B, et al. Amplification of MET may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752. *Proc Natl Acad Sci U S A* 2006;103:2316–21.
74. Rahuel J, Gay B, Erdmann D, et al. Structural basis for specificity of Grb2–2 revealed by a novel ligand binding mode. *Nat Struct Biol* 1996;3:586–9.
75. Dharmawardana PG, Peruzzi B, Giubellino A, Burke TR, Jr., Bottaro DP. Molecular targeting of growth factor receptor-bound 2 (Grb2) as an anti-cancer strategy. *Anticancer Drugs* 2006;17:13–20.
76. Neckers L. Hsp90 inhibitors as novel cancer chemotherapeutic agents. *Trends Mol Med* 2002;8:S55–61.
77. Webb CP, Hose CD, Koochekpour S, et al. The geldanamycins are potent inhibitors of the hepatocyte growth factor/scatter factor-met-urokinase plasminogen activator-plasmin proteolytic network. *Cancer Res* 2000;60:342–9.
78. Xie Q, Gao CF, Shinomiya N, et al. Geldanamycins exquisitely inhibit HGF/SF-mediated tumor cell invasion. *Oncogene* 2005;24:3697–707.
79. Birt AR, Hogg GR, Dube WJ. Hereditary multiple fibrofolliculomas with trichodiscomas and acrochordons. *Arch Dermatol* 1977;113:1674–7.
80. Zbar B, Alvord G, Glenn G, et al. Risk of renal and colon neoplasms and spontaneous pneumothorax in the Birt Hogg Dube syndrome. *Cancer Epidemiol Biomarkers Prev* 2002;11:393–400.
81. Toro J, Duray PH, Glenn GM, et al. Birt-Hogg-Dube syndrome: a novel marker of kidney neoplasia. *Arch Dermatol* 1999;135:1195–202.
82. Pavlovich CP, Grubb RL, Hurley K, et al. Evaluation and management of renal tumors in the Birt-Hogg-Dube syndrome. *J Urol* 2005;173:1482–6.
83. Schmidt LS, Warren MB, Nickerson ML, et al. Birt Hogg Dube syndrome, a genodermatosis associated with spontaneous pneumothorax and kidney neoplasia, maps to chromosome 17p11.2. *Am J Hum Genet* 2001;69:876–82.
84. Nickerson ML, Warren MB, Toro JR, et al. Mutations in a novel gene lead to kidney tumors, lung wall defects, and benign tumors of the hair follicle in patients with the Birt-Hogg-Dube syndrome. *Cancer Cell* 2002;2:157–64.
85. Schmidt LS, Nickerson ML, Warren MB, et al. Germline BHD mutation spectrum and phenotype analysis in a large cohort of families with the Birt-Hogg-Dube syndrome. *Am J Hum Genet* 2005;76:1023–33.
86. Vocke CD, Yang Y, Pavlovich CP, et al. High frequency of somatic frameshift BHD gene mutations in Birt-Hogg-Dube-associated renal tumors. *J Natl Cancer Inst* 2005;97:931–5.
87. Warren MB, Torres-Cabala CA, Turner ML, et al. Expression of Birt-Hogg-Dube gene mRNA in normal and neoplastic human tissues. *Mod Pathol* 2004;17:998–1011.
88. Launonen V, Vierimaa O, Kiuru M, et al. Inherited susceptibility to uterine leiomyomas and renal cell cancer. *Proc Natl Acad Sci U S A* 2001;98:3387–2.
89. Merino MJ, Torres-Cabala CA, Zbar B, Chian-Garcia CA, Linehan WM. Hereditary leiomyomatosis and renal cell carcinoma syndrome (HLRCC): clinical, histopathological and molecular features of the first American families described. *Modern Pathology United States and Canadian Academy of Pathology Meeting Abstracts* 2003;16:739.
90. Toro JR, Nickerson ML, Wei MH, et al. Mutations in the fumarate hydratase gene cause hereditary leiomyomatosis and renal cell cancer in families in North America. *Am J Hum Genet* 2003;73:95–106.
91. Wei M-H, Toure O, Glenn GM, et al. Novel mutations in FH and expansion of the spectrum of phenotypes expressed in families with hereditary leiomyomatosis and renal cell cancer. *J Med Genet* 2006;43:18–27.
92. Tomlinson IP, Alam NA, Rowan NA, et al. and The Multiple Leiomyoma Consortium. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. *Nat Genet* 2002;30:406–10.
93. Isaacs JS, Jung YJ, Mole DR, et al. HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. *Cancer Cell* 2005;8:143–53.
94. Vanharanta S, Pollard PJ, Lehtonen HJ, et al. Distinct expression profile in fumarate-hydratase-deficient uterine fibroids. *Hum Mol Genet* 2006;15:97–103.
95. Kelland LR, Sharp SY, Rogers PM, Myers TG, Workman P. DT-Diaphorase expression and tumor cell sensitivity to 17-allylamino, 17-demethoxygeldanamycin, an inhibitor of heat shock protein 90. *J Natl Cancer Inst* 1999;91:1940–9.
96. Guo W, Reigan P, Siegel D, Zirrolli J, Gustafson D, Ross D. Formation of 17-allylamino-demethoxygeldanamycin (17-AAG) hydroquinone by NAD(P)H:quinone oxidoreductase 1: role of 17-AAG hydroquinone in heat shock protein 90 inhibition. *Cancer Res* 2005;65:10006–15.
97. Fishman MC, Porter JA. Pharmaceuticals: a new grammar for drug discovery. *Nature* 2005;437:491–3.
98. Toro JR, Glenn G, Hou L, et al. Facial papules, spontaneous pneumothorax, and renal tumors. *J Am Acad Dermatol* 2003;48:111–4.