The Role of Hepatocyte Growth Factor Pathway Signaling in Renal Cell Carcinoma

Fabiola Cecchi, Young H. Lee, Benedetta Peruzzi,
Jean-Baptiste Lattouf and Donald P. Bottaro[1]

Urologic Oncology Branch, Center for Cancer Research, National Cancer Institute,
National Institutes of Health, Bethesda, MD 20892 USA

[1]Correspondence:

Urologic Oncology Branch, CCR, NCI
Bldg 10 CRC Rm 2-3852
10 Center Drive MSC 1107
Bethesda, MD 20892-1107 USA

Telephone (301) 402-6499
Fax (301) 402-0922
Email: dbottaro@helix.nih.gov

Running Title: Met Signaling in Renal Cancer

Keywords: Hepatocyte growth factor, Met, renal cell carcinoma, oncogenic mechanisms, cancer drug development

Abstract

The urgent need for effective therapies for patients with advanced renal cell carcinoma (RCC), fewer than twenty percent of whom will survive more than two years, has led to the identification of critical genetic determinants and associated molecular pathways contributing to RCC oncogenesis, progression and spread. Among the signaling pathways dysregulated in RCC is that of hepatocyte growth factor (HGF), which through the cell surface receptor tyrosine kinase, Met, stimulates proliferation, motility and morphogenesis. Germline missense mutations in the tyrosine kinase domain Met are associated with hereditary papillary renal carcinoma (HPRC), while somatic mutations and frequent trisomy of chromosome 7 implicate pathway involvement in sporadic papillary type 1 RCC. In addition, loss of the *VHL* tumor suppressor gene results in the derepression of an embryonic HGF-driven phenotype likely to contribute to tumor invasiveness and metastasis in clear cell RCC. Our knowledge of HGF/Met signaling has enabled rapid progress in characterizing its contributions to RCC and in laying the framework for the development of novel anti-cancer therapeutics. A better understanding of how HGF/Met signaling is integrated with other oncogenic pathways in RCC should aid the development of combinatorial treatment strategies, and help predict potential adverse effects of long-term pathway blockade.

1. Introduction

Over 200,000 cases of kidney cancer are diagnosed each year worldwide, claiming more than 100,000 lives (1). Despite significant advances in the development of immunologic therapies for this disease, there is still no effective therapy for the majority of patients with advanced RCC (1;2). Four main sporadic RCC subtypes with distinct histologies are currently recognized: clear cell, papillary, chromophobe and oncocytoma. Papillary RCC is further sub classified into types 1 and 2 based on additional clinical, histological and genetic criteria (2). Rare, inherited forms of RCC exist which characteristically present with one or two of these histological subtypes; the study of these familial diseases has facilitated the identification of their underlying genetic defects, and helped forge mechanistic links with sporadic RCC types with similar histologies (2). For two prevalent RCC subtypes, clear cell and papillary type 1, these mechanistic links strongly implicate the HGF signaling pathway in oncogenesis, tumor progression and metastasis.

HGF is a plasminogen-like protein with mitogenic, motogenic and morphogenic activities (3;4). HGF is typically produced by cells of mesenchymal origin and acts in a paracrine manner on a variety of cellular targets including epithelial and endothelial cells, hematopoietic cells, neurons, melanocytes as well as hepatocytes (3;4). HGF is essential for early embryonic development and also contributes to organogenesis in liver, lung, kidney and other tissues (5). The cell surface receptor for HGF is the transmembrane tyrosine kinase encoded by the *MET* protoncogene (6). The *MET* oncogene was isolated from a chemically mutagenized human osteogenic sarcoma cell line; its transforming activity was due to gene rearrangement where sequences from the *TPR* (translocated promoter region) locus on chromosome 1 fused to sequences from the *MET* locus on chromosome 7 (*TPR-MET*) (7). Subsequent isolation of the full-length *MET* proto-oncogene coding sequence revealed structural features of a membrane spanning receptor tyrosine kinase (TK) (7). The identification of HGF as the natural ligand for the Met receptor protein and the identity of scatter factor and HGF united a collection of findings demonstrating that a single receptor transduced all HGF biological activities (3). Consistent with its relationship with HGF, *MET* is widely expressed early in

development, deletion of the gene is lethal in mice, and widespread expression persists throughout adulthood (5;8).

2. The HGF/Met Signaling Pathway: an Overview

Upon HGF binding, Met is autophosphorylated on two tyrosine residues (Y1234 and Y1235 per sequence for UniProt accession P08581) within the activation loop of the TK domain which significantly enhance kinase activity, while phosphorylation on two tyrosine residues near the carboxyl terminus (Y1349 and Y1356) form a multifunctional docking site that recruits a collection of intracellular adapters containing Src homology-2 (SH2) domains and other specific receptor recognition motifs that transmit signals further downstream (7;9). Among the adapter proteins and direct kinase substrates thus far implicated in Met signaling are Grb2, Gab1, phosphatidylinositol 3-kinase (PI3K), phospholipase C-gamma (PLCγ), Shc, Src, Shp2, Ship1, and STAT3 (7). Gab1 and Grb2 in particular connect larger networks of adaptor proteins involved in signaling, presumably contributing to HGF pleiotropism (3;7). The direct binding of Grb2 to Met through Y1356 links the receptor to the Ras/MAPK pathway regulating cell cycle progression (3;7;9). Gab1/Met interactions initiate branching morphogenesis in several epithelial and vascular endothelial cell types. Gab1 is also highly phosphorylated by Met, resulting in the recruitment of PI3K, contributing in turn to cell cycle progression, protection from apoptosis, as well as increased cell motility (7-9). Among the many genes upregulated by this pathway is *MET* itself (9), creating the potential for Met overexpression in otherwise normal target cells through persistent ligand stimulation; indeed, Met overexpression is widely observed in cancers of epithelial origin (3).

HGF/Met signaling is implicated in a wide variety of human malignancies including colon, gastric, bladder, breast, kidney, liver, lung, head and neck, thyroid and prostate cancers, sarcomas, hematological malignancies, melanoma and central nervous system tumors (3). Through paracrine signaling, overexpression of ligand and/or receptor, autocrine loop formation and/or receptor mutation and gene rearrangement, this signaling pathway can enhance tumor cell growth, proliferation, survival, motility and invasion (3;7;9). Inappropriate Met signaling in disease can resemble developmental transitions between epithelial and mesenchymal cell types normally regulated by HGF.

Importantly, the pathway initiates a program of cell dissociation and increased cell motility coupled with increased protease production that has been shown to promote cellular invasion through extracellular matrices, and that closely resembles tumor metastasis *in vivo* (9). In addition, pathway activation in vascular cells stimulates tumor angiogenesis, facilitating tumor growth for cancers that are growth limited by hypoxia and promoting tumor metastasis (9). Hypoxia alone upregulates Met expression and enhances HGF signaling in cultured cells and mouse tumor models (9).

3. The HGF/Met Signaling Pathway in Kidney

3.1 HGF Signaling in Kidney Development

The critical roles of HGF and Met in embryonic development were first demonstrated in mice by targeted disruption of each gene; these animals displayed placental defects, defective somite migration, stunted liver and limb muscle development and death *in utero* (5;10). HGF promotes the development of tubular structures in organs such as mammary gland and kidney (11). Proper kidney development depends on the multicellular process of branching morphogenesis. During the metanephric phase of kidney development, nephrogenesis is initiated by ingrowth of the Wolffian duct-derived ureteric bud into the presumptive kidney mesenchyme (11;12). In response to signals from the ureter, mesenchymal cells condense, aggregate into pretubular clusters and undergo an epithelial conversion generating a simple tubule. This tubule then undergoes morphogenesis and is transformed into the excretory system of the kidney. The nephron epithelial tube gives rise to the branched collecting duct system, while the surrounding metanephric mesenchyme undergoes mesenchymal-epithelial transition to form the proximal parts of the nephron (11;12). The coordinated exchange of signals in both directions between the growing buds of epithelium and the mesenchyme that they are invading is critical. Several soluble factors act in a complementary fashion either as pro- or anti-tubulogenic regulators, including members of the fibroblast growth factor, transforming growth factor-beta and Wnt families as well as glial derived neurotrophic factor, epidermal growth factor and HGF (11;12).

The HGF-driven intracellular signaling events in mesenchymal/epithelial transitions during nephrogenesis presumably resemble those defined using cultured renal

epithelial cell models of branching morphogenesis. In that context, the recruitment of Gab1 and Grb2 to c-Met activates SOS1, contributing to Ras–MAP kinase pathway activation, adherens junction disassembly, cell motility and proliferation (13). Reorganization of the actin cytoskeleton, which is required for observed cell shape changes, is regulated by the Rho family of small GTPases activated downstream of PI3K and Ras (13;14). Rac1 and cdc42 regulate actin polymerization at the cell periphery resulting in the extension of lamellipodia that are essential for cell migration and fillopodia that precede *de novo* tubulogenesis *in vitro* (13;14). In contrast, RhoA acting via its downstream effector Rho-associated kinase stimulates myosin light chain phosphorylation and regulates actin stress fiber formation and cell contractility (14). Thus a coordinated activation and deactivation of Rac and Rho is required for cell shape change and migration (11;13;14). HGF stimulation also results in the tyrosyl phosphorylation of β-catenin, inducing its dissociation from E-cadherin in adherens junctions, contributing to junction breakdown and freeing β-catenin for nuclear translocation and transcriptional activation (8).

3.2 HGF Signaling in Renal Homeostasis

HGF and *MET* expression persist in the adult kidney, but striking changes occur in the quality and magnitude of the response of renal epithelial cells to HGF stimulation upon completion of normal development. Morphogenic and proliferative responses are minimized. While the role of HGF in adult renal physiology is not yet fully understood, the kidney is an important source of circulating HGF in adults, and HGF is an endogenous renoprotective factor with potent antifibrotic activity (15;16). HGF has been shown to protect adult kidney tissue from acute toxicity and ischemic stress (15). Endogenous HGF levels are elevated in kidneys exposed to long term stress, and HGF counteracts TGF-β signaling associated with renal fibrosis, a major cause of chronic renal failure (15-17). At the cellular level, these tissue protective effects are most likely to be mediated through HGF-driven cell survival pathways and pathways that control extracellular matrix composition and turnover (15-17).

3.3 Dysregulated HGF Signaling in RCC

Most of the intracellular mediators and pathways activated by Met persist through development into adulthood, and it is unclear which signals are modified or silenced to provide a homeostatic, as opposed to developmental or pathological, HGF response. Given the functional similarities between tumorigenesis and epithelial/mesenchymal transitions at the cellular level, the loss of such signal attenuation mechanisms are likely to contribute to tumorigenesis, invasiveness and metastasis. Among the four main RCC subtypes, an oncogenic role of HGF/Met signaling has been firmly established for hereditary papillary renal carcinoma (HPRC), where inherited missense mutations in the *MET* gene were first found; similar somatic mutations were also found in a small subset (13%) of sporadic papillary renal carcinoma (PRC) tumor samples (18-21). The biochemical and biological impact of these *MET* mutants have been investigated in several model systems, confirming their suspected oncogenic potential, as described in greater detail below (22-28). A growing body of evidence also supports HGF/Met pathway involvement in clear cell RCC, where loss of *von Hippel-Lindau* (*VHL*) tumor suppressor gene function occurs in familial and most sporadic cases (2). *VHL* loss results in the aberrant expression of genes that control cell proliferation, invasion and angiogenesis (2).

3.3.1 HGF/Met Pathway Activation in HPRC and Sporadic PRC Type 1

Several missense mutations in *MET* have been identified in individuals with PRC Type 1, HPRC, in other human cancers, as well as in cancer cell lines (21). Schmidt, *et al.* first reported nucleotide changes in exons 17, 18 and 19 in the germlines of HPRC families and also in a subset of sporadic papillary renal carcinomas (18). Five germ line mutations and four somatic mutations were localized to the Met TK domain (18). Of the five germline mutations found, D1246H and D1246N were located in the codon homologous to a naturally occurring mutation in c-kit, which is responsible for systemic mastocytosis in humans (18). Another mutation, M1268T, was homologous in position and residue change to the human *RET* proto-oncogene codon mutated in multiple endocrine neoplasia (MEN) type 2B and sporadic medullary carcinoma of the thyroid gland (18). Later studies revealed a germ line mutation in exon 16 of (H1112R), which significantly enhanced focus formation when ectopically expressed in NIH3T3 cells, and

V1110I, a mutation also found in the homologous codon (V157I) of chicken *c-erbB*, where it triggers the sarcomagenic potential of the *v-erbB* oncogene (19;20;29).

The biochemical and biological impact of these *MET* mutants were first investigated in NIH3T3 cell transfectants (22;23;30). Mutant Met receptors displayed increased levels of tyrosyl autophosphorylation relative to wild type (WT) receptors, as well as greater TK activity towards an exogenous substrate (22;30). Cells expressing mutant receptors acquired focus forming activity in monolayer culture, and the ability to form tumors in athymic nude mice, in contrast to weak tumorigenicity displayed by WT Met in the same context (22;23;30). Mutant receptors showed increased cell motility relative to WT, as well as increased intracellular activation of the Ras-Raf-MEK-ERK signaling pathway (23;24;30). Transgenic mice harboring the PRC mutant Met constructs under the control of a metallothionein promoter developed metastatic mammary carcinoma, confirming that these *MET* mutations were oncogenic (22;23;30).

Later analysis of an extended panel of tumor samples included the complete sequencing of exons 5 and 7 in the extracellular domain, exon 13 encoding the transmembrane domain, and exons 14-20 encoding the bulk of the intracellular portion of the receptor (19). These studies showed that *MET* mutations occur in only a small proportion (13%) of sporadic PRC, which is noteworthy in light of prior reports of highly frequent (95%) trisomy of chromosome 7 in this disease (31). A detailed study of trisomy 7 in HPRC showed that duplication of the mutant *MET* allele occurred in 16 of 16 tumor samples, suggesting that having two copies of the mutant allele conferred a proliferative advantage to the affected tumor cells (32). While this potential mechanism of selective overexpression of mutant Met can be viewed as providing a "second hit" leading to tumorigenesis, the prevalence of trisomy 7 in sporadic PRC indicates that most PRC tumors display trisomy 7 in the absence of *MET* mutations (31;32). Whether the potentially increased dose of *MET* and/or HGF genes, both located on chromosome 7, confers a selective advantage in the absence of mutation is an intriguing hypothesis that warrants further investigation.

Several studies have addressed in detail the mechanisms by which PRC-associated *MET* mutations act at the cellular and molecular levels. Bardelli, *et al*. showed that the M1268T mutation changed substrate preference *in vitro*, using a panel of peptides

differentially phosphorylated by epidermal growth factor receptor (EGFR), Src, or Abl; M1268T acquired a preference similar to that displayed by the homologous *RET* mutation characteristic of MEN 2B (24). When expressed in NIH3T3 cells, the mutations Y1248H, D1246H/N and M1268T showed constitutive association with the key intracellular effector Gab1 (24). Similar to signaling by WT Met, the link to Gab1 and other effectors required phosphorylation of the carboxyl-terminal docking sites, as did other indices of cell transformation such as growth in soft agar (24). Thus the oncogenicity of Met mutants is mediated by many of the same receptor-proximal intracellular effectors involved in WT Met signaling, suggesting that interruption of key receptor-effector interactions at the carboxyl-terminal docking sites remains a viable strategy for blocking mutant Met signaling (24).

Building upon prior studies, Giordano, *et al.* hypothesized that different mutations may contribute to disease pathogenesis through distinct molecular pathways downstream of Met (25). When ectopically expressed in NIH3T3 cells or the murine liver oval cell line MLP 29, the *MET* PRC mutants fell into two functional groups: M1268T and D1246H possessed enhanced receptor kinase activity, stimulated increased Ras pathway activation and transformed cells in focus formation assays (25). Mutations L1213V and Y1248C, in contrast, displayed lower kinase activity, Ras pathway activation and focus forming ability, but were more effective in PI3K pathway activation, protecting cells from apoptosis, sustaining soft agar colony formation and promoting matrix invasion (25). All of these effects were enhanced upon addition of HGF (25).

The role of ligand binding in the oncogenic potential of PRC-associated *MET* mutations was investigated further by Michieli, *et al*. using cultured epithelial cells, which typically do not express HGF (26). Met mutants reconstituted in MDCK epithelial cells required exogenously added ligand for colony formation in soft agar (26). Met mutants reconstituted in truncated receptor constructs lacking most of the extracellular domain failed to induce focus formation, and M1268T reconstituted in this context was transforming only upon addition of a receptor-ligating monoclonal antibody (26). Soft agar colony formation by NIH3T3 cells bearing Met M1268T could be blocked by coexpression of a soluble Met extracellular domain, an uncleavable form of HGF, or the HGF competitive antagonist HGF/NK4 (26). Together these results revealed that ligand

binding contributes significantly to oncogenesis associated with PRC *MET* mutations. Ligand dependence may explain why patients with germline *MET* mutations exhibit only kidney cancer, as the kidney is an abundant source of HGF, as well as urokinase, an important activator of immature HGF. Michieli, *et al*. speculated that the long term combination of ligand, ligand activator, and highly responsive target cells may render these otherwise benign receptor mutations "regionally" oncogenic (26).

In the first study designed to predict how PRC-associated *MET* mutations might alter catalytic function, Miller, *et al.* aligned the TK domain of Met with that of the insulin receptor by computer modeling (27). The results showed that certain HPRC mutations could disrupt the normal mechanism of TK autoinhibition, thereby stabilizing the active form of the receptor (27). In the unphosphorylated form of the WT receptor, residues in the activation loop of the TK domain normally block access to ATP and to peptide substrates, while phosphorylation of specific tyrosine residues leads to stabilization of the open, active conformation (27-29). Notably, M1268T and Y1248C/D/H were predicted to stabilize the open, active TK conformation. Mutation of Y1248 to the more hydrophilic residues C, D, or H might also stabilize the active TK conformation by rendering the site resistant to phosphatase action (27). Overall, these findings predicted that mutant Met forms are more easily activated than WT Met, and more likely to remain active, but did not that clearly eliminate the need for an initiator of kinase activation such as ligand binding or other environmental cue.

In a study complementary to that of Miller, *et al.,* Chiara and colleagues later compared the autophosphorylation events in WT and mutant Met receptors expressed in cultured cells using phosphorylation-site specific antibodies, and proposed that mutant receptors possessed a lower threshold for kinase activation (30). HGF binding to WT Met triggers autophosphorylation of Y1235 and Y1234 in the TK activation loop; substitution of F for Y at either position severely impairs kinase function, suggesting that phosphorylation at both sites is required for kinase activation (30,31) A more recent study further showed that mutation in Y1235D reduced k_{cat} compared with the activated, autophosphorylated wild-type enzyme (32). Unlike WT Met, the D1246H/N and M1268T Met mutants did not undergo Y1234 phosphorylation, and were not catalytically impaired by F substitutions at that site (30). Thus these mutants were not constitutively active, but

mutation overcame the normal requirement for a second phosphorylation step leading to kinase activation (30). Importantly, the apparent need for ligand activation of HPRC and PRC associated Met mutant forms suggests that therapeutic strategies aimed at ligand blockade remain viable possibilities for these patient populations.

3.3.2 HGF/Met Signaling in Clear Cell RCC

von Hippel-Lindau (VHL) syndrome is an autosomal dominant hereditary neoplastic disorder (3;33;34). VHL-associated clear cell renal cell carcinoma (RCC) tumors are malignant and frequently metastatic (3;34). Defects in the *VHL* tumor suppressor gene, which is located on the short arm of chromosome 3 (3p25-26), lead to VHL syndrome and also occur in the majority of sporadic clear cell RCC cases (3;35). Reconstitution of WT *VHL* expression in RCC derived cells regulates tumorigenesis in mice, confirming a critical role for *VHL* in RCC (36). The VHL protein (pVHL) is part of an E3 ubiquitin ligase complex that targets hypoxia inducible factors for polyubiquitination and proteosomal degradation (37). During hypoxia or when pVHL function is lost, hypoxia inducible factors accumulate and cause broad changes in gene expression that are potentially important in oncogenesis (34;37;38). Cultured *VHL*-negative RCC cells also acquire an abnormal response to HGF, manifested as matrix degradation, increased cell motility, matrix invasion and morphogenesis (39). These HGF driven activities are abolished when WT *VHL* expression is reconstituted in RCC cells, directly linking loss of *VHL* function to an invasive tumor phenotype (39).

Investigating the molecular mechanism by which RCC cells acquire an invasive response to HGF, Peruzzi, *et al.* hypothesized that *VHL* loss in clear cell RCC might promote oncogenic signaling downstream of Met (40). Among the known intracellular mediators of HGF signaling with oncogenic potential is β-catenin, which links cadherins to the actin cytoskeleton and also functions as a gene transactivator (41-44). β-catenin and E-cadherin are initially expressed during renal development, specifically upon transition of the mesenchyme surrounding the branching ureteric buds to the epithelium that will form the tubules of the nephron (45). As described above, this mesenchymal to epithelial transition and ensuing tubule formation involves several Wnt family members acting in an autocrine manner (12;46), as well as HGF acting in a paracrine mode (47).

Dysregulated β-catenin signaling in the adult is potently oncogenic: mutations in the genes encoding APC or β-catenin are frequent in colorectal cancer (48). Both types of mutation allow β-catenin to bypass APC-mediated ubiquitination and proteosomal degradation and it is now known that cytoplasmic β-catenin can be stabilized by a variety of genetic defects (48).

Using several RCC cell models, Peruzzi, *et al.* found that HGF stimulated β-catenin tyrosyl phosphorylation, adherens junction disruption, cytoplasmic β-catenin accumulation and reporter gene transactivation (40). These activities were repressed when *VHL* expression was reconstituted ectopically (40). Expression of a ubiquitination resistant β-catenin mutant specifically restored HGF stimulated invasion and morphogenesis in *VHL* transfected RCC cells, while *VHL* gene silencing in non-RCC renal epithelial cells phenotypically mimicked *VHL* loss in RCC (40). Finally, HGF driven invasiveness was blocked by the expression of a dominant negative mutant of Tcf, reinforcing the conclusion that in RCC cells, *VHL* loss enables HGF-driven oncogenic β-catenin signaling (40). A later report demonstrated that Jade-1, acting downstream of VHL, binds the oncoprotein-β-catenin directly in Wnt-responsive fashion (49). Interestingly, VHL-mediated ubiquitylation of Jade-1 stabilizes the Jade-1 protein, and VHL loss in clear cell RCC is accompanied by Jade-1 loss through proteasomal degradation (50). In the presence of VHL, Jade-1 ubiquitylates both phosphorylated and non-phosphorylated-β-catenin and therefore regulates canonical Wnt signaling in both Wnt-off and Wnt-on phases (49). pVHL downregulates β-catenin in a Jade-1-dependent manner and inhibits Wnt signalling, supporting a role for Jade-1 loss and Wnt signaling in renal tumorigenesis (49). Together these findings identify β-catenin as a potential target for drug development for *VHL*-negative clear cell RCC.

Independent of its role in HGF/Met signaling, the widespread involvement of β-catenin in human cancers has prompted several other investigations of its potential dysregulation in RCC. Initial studies suggest that activating mutations in *β-catenin* are rare in RCC tumors (51-53), and no inactivating mutations in *APC* or *axin* have been reported in RCC (54;55). Oncogenic β-catenin signaling can also be initiated through aberrant Wnt stimulation or loss of negative repressors of Wnt signaling, such as

members of the Dkk family. A recent study demonstrated the striking downregulation of REIC/Dkk-3 in 15 of 17 (88%) RCC tumor samples (56). Further evidence for the activation of Wnt signaling pathway in RCC comes from a recent article (57) which describes the homozygous deletion of CXXC4, a gene coding for Idax (an inhibitor of Wnt signaling) in aggressive RCC. The secreted-Frizzled receptor proteins, Dickkopf 2 and Wnt inhibitory factor 1 are Wnt antagonists and expression of these genes is also silenced by aberrant hypermethylation in RCC (58-61). The persistent expression of Wnt family members from kidney development through adulthood (5;12;62) suggests that loss of this potential Wnt inhibitor combined with the frequent loss of *VHL* function in clear cell RCC could contribute significantly to tumorigenesis, invasion and metastasis.

Xp11 translocation RCC is a newly identified RCC variants added to the WHO 2004 classification (63). The *ASPL-TFE3* fusion arising from a t(X;17)(p11.2;q25.3) characterizes a subset of pediatric renal adenocarcinomas (64). Tsuda et al. [65] discovered that ASPL-TFE3 binds to the *MET* promoter and activates it. Induction of *MET* by ASPL-TFE3 results in an apparent increase in Met protein autophosphorylation and activation of downstream signaling in the presence of HGF. In malignant cell lines containing endogenous TFE3 fusion proteins, inhibiting *MET* expression by RNA interference or inhibition of Met protein by the inhibitor PHA665752 abolishes HGF-dependent Met activation, resulting in decreased cell growth. Met may therefore be an additional therapeutic target in tumors with *TFE3* fusions and these results provide a rationale for clinical trials of Met-targeted therapy in this tumor group [65].

4. Cancer Drug Development: Targeting the HGF/Met Pathway

Our present understanding of oncogenesis mediated by Met signaling supports at least three avenues of therapeutic development: antagonism of ligand/receptor interaction, inhibition of TK catalytic activity, and blockade of receptor/effector interactions (66). In addition, combinations of conventional and Met targeted therapies may offer promise for specific cancers (67).

Antagonism of ligand binding is a logical therapeutic strategy for a majority of carcinomas where paracrine HGF signaling and Met overexpression result in aberrant pathway activation, including PRC and clear cell RCC. Agents currently under

development as HGF/Met pathway inhibitors directed against ligand-receptor binding include competitive molecular analogs of HGF, decoy Met and monoclonal antibodies directed against either HGF or Met. A collection of structure/function studies, including the early discovery that a naturally occurring truncated HGF variant, HGF/NK2, was a specific competitive mitogenic antagonist, led the development of HGF/NK4, a larger, more completely antagonistic HGF fragment (68), and to an uncleavable form of pro-HGF (69), both of which block tumor growth and metastasis in animal models. Similarly, the early development of a Met ectodomain/IgG fusion protein with HGF neutralizing activity preceded the engineering of a soluble Met ectodomain fragments with pathway neutralizing and anti-tumor activities (70;71). Neutralizing mouse monoclonal antibodies against human HGF have also been shown to be effective anti-tumor agents in animal models (72-74). Rilotumumab (AMG 102) is a fully human monoclonal antibody with HGF-neutralizing activity. It was evaluated in a phase II clinical study including patients with all histologic subtypes of advanced RCC and did not select patients based on evidence of Met pathway activation. Only a single partial response was seen in the 61 patients treated at two dose-levels (75). Although rilotumumab is unlikely to offer clinical benefit as a single agent in unselected patients, further evaluation of Met pathway antagonists in tumors with known pathway activation is warranted (NCT00422019).

Recent successes in the treatment of cancers using TK inhibitors strongly support the potential efficacy this therapeutic strategy for targeting Met in RCC. Early work with the non-selective staurosporine-like alkaloid K252a showed that it could inhibit Met autophosphorylation, MAPK and Akt activation, and revert the transforming potential of the *TPR-MET* oncogene (76). Other early TK antagonists that exhibited more selective, but by no means exclusive, activity against Met, such as SU11274 and PHA665752 showed similar preclinical anti-oncogenic potential and revealed that HPRC-associated Met mutations could impact drug sensitivity (77-79). More selective and potent synthetic inhibitors of Met ATP binding have been developed and tested in various model systems (78-79). Most Met TKIs competitively antagonize occupancy of the intracellular ATP binding site, preventing TK activation and downstream signaling. Among these, foretinib targets Met, VEGFR2, Axl, Ron and Tie-2 with high affinity. In the largest clinical trial devoted to papillary renal cell carcinoma, foretinib demonstrated anti-tumor activity,

modulation of several target indicator plasma proteins, and a manageable toxicity profile (80). Unlike previous trials of Met pathway antagonists, this trial was restricted to patients with papillary histology (both type 1 and 2 histologies were included). In addition, patients enrolled on this trial were stratified based on the presence of indications of Met pathway activation to determine if Met status impacted response to the agent (80). The overall response rate in the trial was 13.5%, and the median duration of response was 18.5 months. The median progression-free survival (PFS) was 9.6 months for the whole study population. When analyzed by dosing cohort, the intermediate dosing group treated with 240 mg/day on days 1 to 5 of a 14-day cycle had a slightly longer progression-free survival (PFS) at 11.6 months than patients treated with continuous dosing of 80 mg/day at 9.1 months. Fifty out of the 68 evaluable patients had some degree of tumor shrinkage, although most did not meet the criteria for partial response by RECIST. Remarkably, 5 out of 10 patients with germline *MET* mutations had a partial response. The other five had stable disease for at least six weeks, and four of them had more than 10% tumor shrinkage but less than the 20% necessary for a partial response (81).

Tivantinib is the only Met-directed TK inhibitor currently in human clinical trials that is not ATP-competitive; it reportedly binds to the Met TK domain near the ATP binding site and acts allosterically (82). A phase II, multicenter, single-arm study assessing the safety and efficacy of tivantinib monotherapy in adolescent and adult patients with metastatic or surgically unresectable microphthalmia transcription factor (MITF)-associated (MiT) tumors, including translocation-associated RCC (tRCC), was recently completed. Median progression-free survival was 1.9 months in tRCC and tivantinib was safe and tolerable in patients with MiT tumors, but antitumor activity was modest (83). A randomized phase II clinical trial is recruiting patients with metastatic or locally advanced kidney cancer that cannot be removed by surgery. The primary objective is to assess the response rate (confirmed complete and partial response) of patients with locally advanced or metastatic pRCC treated with either tivantinib or tivantinib combined with erlotinib hydrochloride (NCT01688973).

A cross-tumoral phase II clinical study is recruiting patients to study the antitumor activity of crizotinib across predefined tumor types harboring specific alterations in ALK and/or Met. One arm of the study will test crizotinib in PRC type 1 at doses of 500, 400

or 250 mg/day, depending on toxicity. A phase I/II multiple ascending dose study of BMS-777607 in subjects with advanced or metastatic gastroesophageal cancer, hormone refractory prostate cancer, head and neck squamous cell carcinoma, and PRC type 1 has been completed and results are awaited (NCT00605618). Preliminary analysis of an ongoing phase I clinical trial testing cabozantinib in 25 patients with metastatic clear cell renal cancer showed a median progression-free survival (PFS) of 14.7 months. Of 21 patients evaluable for response, seven had a partial response by modified RECIST criteria, 13 had stable disease and one had progressive disease. Interestingly, the investigators saw responses in patients who had prior anti-VEGF therapy, suggesting that the combination of Met-VEGF inhibition is therapeutically valuable. Further trials in this disease setting are planned (NCT01100619) (84,85)

The requirement of the carboxyl-terminal docking site for WT or mutant Met transforming activity in cultured cells (24;25), and the known roles of intracellular effectors including Gab1, PI3K, Grb2, Shc and STAT3 in cell transformation (4;7), suggest that targeting one or more of these interactions could effectively disrupt Met driven oncogenesis. Knowledge of the unique structure of the Grb2 SH2 domain provided the basis for the development of small synthetic Grb2 selective binding antagonists (86). Further refinement of these early structures has yielded compounds that block HGF-stimulated cell motility, matrix invasion and morphogenesis in normal and tumor derived cultured cells, as well as vascular endothelial cells, at low nanomolar concentrations (87). Beyond effector targeting, compounds that block HSP90/client interactions, such as geldanomycin (88), also potently block Met oncogenic signaling (89,90). Human clinical trials of geldanomycin related compounds are underway for a variety of cancers where the Met pathway is active, including RCC.

While the potential efficacy of HGF/Met targeted drugs for treating subtypes of RCC as single agents is promising, combining agents such as geldanomycin that attenuate receptor supply with inhibitors of other critical receptor functions could lower the effective dose of each, reducing drug toxicity as well as the emergence of drug resistant mutations. Improving our understanding of the molecular basis of oncogenic HGF/Met signaling in RCC should facilitate the development of other combinatorial treatment strategies, and help overcome other challenges facing drug development, such

as identifying patients most likely to benefit from HGF/Met targeted therapeutics, assessing drug activities in tumor tissues, and predicting the potential toxicity of long-term pathway blockade.

Acknowledgements

This work was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

References

(1) Jemal A, Clegg LX, Ward E et al. Annual report to the nation on the status of cancer, 1975-2001, with a special feature regarding survival. Cancer 2004; 101(1):3-27.

(2) 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(18):6282S-66289.

(3) Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, Metastasis, Motility And More. Nat Rev Mol Cell Biol 2003; 4(12):915-925.

(4) Corso S, Comoglio PM, Giordano S. Cancer therapy: can the challenge be MET? Trends Mol Med 2005; 11(6):284-292.

(5) Birchmeier C, Gherardi E. Developmental roles of HGF/SF and its receptor, the c-Met tyrosine kinase. Trends Cell Biol 1998; 8:404-410.

(6) Bottaro DP, Rubin JS, Faletto DL et al. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. Science 1991; 251(4995):802-804.

(7) Zhang YW, Vande Woude GF. HGF/SF-met signaling in the control of branching morphogenesis and invasion. J Cell Biochem 2003; 88(2):408-417.

(8) Rosario M, Birchmeier W. How to make tubes: signaling by the Met receptor tyrosine kinase. Trends Cell Biol 2003; 13(6):328-335.

(9) Boccaccio C, Comoglio PM. Invasive growth: a MET-driven genetic programme for cancer and stem cells. Nat Rev Cancer 2006; 6(8):637-645.

(10) Uehara Y, Minowa O, Mori C et al. Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. Nature 1995; 373(6516):702-705.

(11) Karihaloo A, Nickel C, Cantley LG. Signals Which Build a Tubule. Nephron Exp Nephrol 2005 2005; 100:40-45.

(12) Perantoni AO. Renal development: perspectives on a Wnt-dependent process. Semin Cell Dev Biol 2003; 14(4):201-208.

(13) Potempa S, Ridley AJ. Activation of both MAP kinase and phosphatidylinositide 3-kinase by Ras is required for hepatocyte growth factor/scatter factor-induced adherens junction disassembly. Mol Biol Cell 1998; 9(8):2185-2200.

(14) Ridley AJ, Comoglio PM, Hall A. Regulation of scatter factor/hepatocyte growth factor responses by Ras, Rac, and Rho in MDCK cells. Mol Cell Biol 1995; 15(2):1110-1122.

(15) Matsumoto K, Nakamura T. Hepatocyte growth factor: Renotropic role and potential therapeutics for renal diseases. Kidney International 2001; 59(6):2023-2038.

(16) Liu Y. Hepatocyte growth factor and the kidney. Curr Opin Nephrol Hypertens 2002; 11:23-30.

(17) Liu Y. Renal fibrosis: new insights into the pathogenesis and therapeutics. Kidney Int 2006; 69(2):213-217.

(18) Schmidt L, Duh FM, 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(1):68-73.

(19) Schmidt L, Junker K, Nakaigawa N et al. Novel mutations of the MET proto-oncogene in papillary renal carcinomas. Oncogene 1999; 18(14):2343-2350.

(20) Olivero M, Valente G, Bardelli A et al. Novel mutation in the ATP-binding site of the MET oncogene tyrosine kinase in a HPRCC family. Int J Cancer 1999; 82(5):640-643.

(21) Dharmawardana PG, Giubellino A, Bottaro DP. Hereditary papillary renal carcinoma type I. Curr Mol Med 2004; 4(8):855-868.

(22) 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(21):11445-11450.

(23) Jeffers M, Fiscella M, Webb CP, Anver M, Koochekpour S, Vande Woude GF. The mutationally activated Met receptor mediates motility and metastasis. Proc Natl Acad Sci U S A 1998; 95(24):14417-14422.

(24) 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(24):14379-14383.

(25) Giordano S, Maffe A, Williams TA et al. Different point mutations in the met oncogene elicit distinct biological properties. FASEB J 2000; 14(2):399-406.

(26) 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(37):5221-5231.

(27) 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 2001; 44(1):32-43.

(28) Wang W, Marimuthu A, Tsai J et al. Structural characterization of autoinhibited c-Met kinase produced by coexpression in bacteria with phosphatase. Proc Natl Acad Sci U S A. 2006;103(10):3563-3568

(29) Rickert KW, Patel SB, Allison TJ et al. Structural basis for selective small molecule kinase inhibition of activated c-Met. J Biol Chem. 2011; 286(13):11218-11225

(30) 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(31):29352-29358.

(31) Schiering N, Knapp S, Marconi M et al. Crystal structure of the tyrosine kinase domain of the hepatocyte growth factor receptor c-Met and its complex with the microbial alkaloid K-252a. Proc Natl Acad Sci U S A. 2003; 100(22):12654-9.

(32) Timofeevski SL, McTigue MA, Ryan K et al. Enzymatic characterization of c-Met receptor tyrosine kinase oncogenic mutants and kinetic studies with aminopyridine and triazolopyrazine inhibitors. Biochemistry. 2009;48(23):5339-5349.

(33) Latif F, Tory K, Gnarra J et al. Identification of the von Hippel-Lindau disease tumor suppressor gene. Science 1993; 260 (5112):1317-1320.

(34) Linehan WM, Walther MM, Zbar B. The genetic basis of the cancer of the kidney. J Urol 2003; 170:2163-2172.

(35) Gnarra JR, Tory K, Weng Y et al. Mutations of the VHL tumour suppressor gene in renal carcinoma. Nat Genet 1994; 7(1):85-90.

(36) Iliopoulos O, Kibel A, Gray S, Kaelin WG, Jr. Tumour suppression by the human von Hippel-Lindau gene product. Nat Med 1995; 1(8):822-826.

(37) Kaelin WG Jr. Molecular basis of the VHL hereditary cancer syndrome. Nat Rev Cancer 2002; 2(9):673-682.

(38) Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 2003; 3(4):347-361.

(39) Koochekpour S, Jeffers M, Wang PH et al. The von Hippel-Lindau Tumor Suppressor Gene Inhibits Hepatocyte Growth Factor/Scatter Factor-Induced Invasion and Branching Morphogenesis in Renal Carcinoma Cells. Mol Cell Biol 1999; 19(9):5902-5912.

(40) Peruzzi B, Athauda G, Bottaro DP. The von Hippel-Lindau tumor suppressor gene product represses oncogenic beta-catenin signaling in renal carcinoma cells. Proc Natl Acad Sci U S A 2006; 103(39):14531-14536.

(41) Shibamoto S, Hayakawa M, Takeuchi K et al. Tyrosine phosphorylation of beta-catenin and plakoglobin enhanced by hepatocyte growth factor and epidermal growth factor in human carcinoma cells. Cell Adhes Commun 1994; 1(4):295-305.

(42) Papkoff J, Aikawa M. WNT-1 and HGF Regulate GSK3[beta] Activity and [beta]-Catenin Signaling in Mammary Epithelial Cells. Biochemical and Biophysical Research Communications 1998; 247(3):851-858.

(43) Monga SP, Mars WM, Pediaditakis P et al. Hepatocyte Growth Factor Induces Wnt-independent Nuclear Translocation of beta-Catenin after Met-beta-Catenin Dissociation in Hepatocytes. Cancer Research 2002; 62(7):2064-2071.

(44) Herynk MH, Tsan R, Radinsky R, Gallick GE. Activation of c-Met in colorectal carcinoma cells leads to constitutive association of tyrosine-phosphorylated beta-catenin. Clin Exp Metastasis 2003; 20(4):291-300.

(45) Huber SM, Braun GS, Segerer S, Veh RW, Horster MF. Metanephrogenic mesenchyme-to-epithelium transition induces profound expression changes of ion channels. Am J Physiol Renal Physiol 2000; 279(1):F65-F76.

(46) Vainio SJ. Nephrogenesis regulated by Wnt signaling. J Nephrol 2003; 16(2):279-285.

(47) van Adelsberg J, Sehgal S, Kukes A et al. Activation of hepatocyte growth factor (HGF) by endogenous HGF activator is required for metanephric kidney morphogenesis in vitro. J Biol Chem 2001; 276(18):15099-15106.

(48) Polakis P. Wnt signaling and cancer. Genes Dev 2000; 14(15):1837-1851.

(49) Chitalia VC, Foy RL, Bachschmid MM et al. Jade-1 inhibits Wnt signalling by ubiquitylating beta-catenin and mediates Wnt pathway inhibition by pVHL. Nat Cell Biol. 2008 Oct;10(10):1208-16.

(50) Zhou MI, Foy RL, Chitalia VC et al. Jade-1, a candidate renal tumor suppressor that promotes apoptosis. Proc Natl Acad Sci U S A. 2005 Aug 2;102(31):11035-40.

(51) Bilim V, Kawasaki T, Katagiri A, Wakatsuki Sj, Takahashi K, Tomita Y. Altered Expression of beta-Catenin in Renal Cell Cancer and Transitional Cell Cancer with the Absence of beta-catenin Gene Mutations. Clin Cancer Res 2000; 6(2):460-466.

(52) Kim YS, Kang YK, Kim JB, Han SA, Kim KI, Paik SR. beta-catenin expression and mutational analysis in renal cell carcinomas. Pathol Int 2000; 50(9):725-730.

(53) Zhu X, Kanai Y, Saito A, Kondo Y, Hirohashi S. Aberrant expression of beta-catenin and mutation of exon 3 of the beta-catenin gene in renal and urothelial carcinomas. Pathology International 2000; 50(12):945-952.

(54) Bohm M, Wieland I, Stinhofer C, Otto T, Rubben H. Detection of loss of heterozygosity in the APC tumor suppressor gene in nonpapillary renal cell carcinoma by microdissection and polymerase chain reaction. Urol Res 1997; 25(3):161-165.

(55) Suzuki H, Ueda T, Komiya A et al. Mutational state of von Hippel-Lindau and adenomatous polyposis coli genes in renal tumors. Oncology 1997; 54(3):252-257.

(56) Kurose K, Sakaguchi M, Nasu Y et al. Decreased expression of REIC/Dkk-3 in human renal clear cell carcinoma. J Urol 2004; 171(3):1314-1318.

(57) Kojima T, Shimazui T, Hinotsu S et al. Decreased expression of CXXC4 promotes a malignant phenotype in renal cell carcinoma by activating Wnt signaling. Oncogene 2009; 28:297-305.

(58) Hirata H, Hinoda Y, Nakajima K et al. Wnt antagonist gene DKK2 is epigenetically silenced and inhibits renal cancer progression through apoptotic and cell cycle pathways. Clin Cancer Res 2009; 15:5678-87.

(59) Hirata H, Hinoda Y, Nakajima K et al. Wnt antagonist gene polymorphisms and renal cancer. Cancer 2009; 115:4488-503.

(60) Kawakami K, Hirata H, Yamamura S et al. Functional significance of Wnt inhibitory factor-1 gene in kidney cancer. Cancer Res 2009; 69:8603-10.

(61) Urakami S, Shiina H, Enokida H et al. Epigenetic inactivation of Wnt inhibitory factor-1 plays an important role in bladder cancer through aberrant canonical Wnt/beta-catenin signaling pathway. Clin Cancer Res 2006; 12:383-91.

(62) Surendran K, Simon TC, Liapis H, McGuire JK. Matrilysin (MMP-7) expression in renal tubular damage: association with Wnt4. Kidney Int 2004; 65(6):2212-2222.

(63) Eble, JN.; Sauter, G.; Epstein, JI., et al. World health organization classification of tumours. Lyon, France: IARC Press; 2004. Pathology and genetics of tumours of the genitourinary system and male genital organs.

(64) Argani P, Hawkins A, Griffin CA, et al. A distinctive pediatric renal neoplasm characterized by epithelioid morphology, basement membrane production, focal hmb45 immunoreactivity, and t(6;11)(p21.1;q12) chromosome translocation. Am J Pathol. 2001; 158(6):2089–2096.

(65) Tsuda M, Davis IJ, Argani P, et al. TFE3 fusions activate MET signaling by transcriptional up-regulation, defining another class of tumors as candidates for therapeutic MET inhibition. Cancer Res. 2007;67:919–29.

(66) Peruzzi B, Bottaro DP. Targeting the c-Met signaling pathway in cancer. Clin Cancer Res 2006; 12(12):3657-3660.

(67) Aebersold 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(52):8519-8523.

(68) Matsumoto K, Nakamura T. Mechanisms and significance of bifunctional NK4 in cancer treatment. Biochemical and Biophysical Research Communications 2005; 333(2):316-327.

(69) Mazzone M, Comoglio PM. The Met pathway: master switch and drug target in cancer progression. FASEB J 2006; 20(10):1611-1621.

(70) 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(1):61-73.

(71) Kong-Beltran M, Seshagiri S, Zha J et al. Somatic Mutations Lead to an Oncogenic Deletion of Met in Lung Cancer. Cancer Research 2006; 66(1):283-289.

(72) 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. PNAS 2001; 98(13):7443-7448.

(73) Martens T, Schmidt N-O, Eckerich C et al. Inhibition of intracerebral glioblastoma growth by treatment with a novel one-armed anti-Met antibody. GMS/German Medical Science, an e-journal . 5-4-2005.

(74) 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(4):1292-1298.

(75) Schoffski P, Garcia JA, Stadler WM, et al. A phase ii study of the efficacy and safety of amg 102 in patients with metastatic renal cell carcinoma. BJU Int. 2011; 108(5):679–686.

(76) Morotti A, Mila S, Accornero P, Tagliabue E, Ponzetto C. K252a inhibits the oncogenic properties of Met, the HGF receptor. Oncogene 2002; 21(32):4885-4893.

(77) Berthou S, Aebersold DM, Schmidt LS et al. The Met kinase inhibitor SU11274 exhibits a selective inhibition pattern toward different receptor mutated variants. Oncogene 2004; 23(31):5387-5393.

(78) Christensen JG, Burrows J, Salgia R. c-Met as a target for human cancer and characterization of inhibitors for therapeutic intervention. Cancer Letters 2005; 225(1):1-26.

(79) 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. PNAS 2006; 103(7):2316-2321.

(80) Choueiri TK, Vaishampayan U, Rosenberg JE et al. Phase II and Biomarker Study of the Dual MET/VEGFR2 Inhibitor Foretinib in Patients With Papillary Renal Cell Carcinoma. J Clin Oncol 2013; 31(2):181-6.

(81) Srinivasan R, Bottaro DP, Choueiri TK et al. Correlation of germline MET mutation with response to the dual Met/VEGFR-2 inhibitor foretinib in patients with sporadic and hereditary papillary renal cell carcinoma: Results from a multicenter phase II study (MET111644). J Clin Oncol 30, 2012 (suppl 5; abstr 372).

(82) Munshi N, Jeay S, Li Y, Chen CR et al. ARQ 197, a novel and selective inhibitor of the human c-Met receptor tyrosine kinase with antitumor activity. Mol Cancer Ther. 2010 Jun;9(6):1544-53.

(83) Wagner AJ, Goldberg JM, Dubois SG, Choy E, Rosen L, Pappo A, Geller J, Judson I, Hogg D, Senzer N, Davis IJ, Chai F, Waghorne C, Schwartz B, Demetri GD. Tivantinib (ARQ 197), a selective inhibitor of MET, in patients with microphthalmia transcription factor-associated tumors: results of a multicenter phase 2 trial. Cancer. 2012 Dec 1;118(23):5894-902.

(84) Choueiri TK, Pal SK, McDermott DF et al. Activity of cabozantinib (XL184) in patients (pts) with metastatic, refractory renal cell carcinoma (RCC). J Clin Oncol 30, 2012 (suppl 5; abstr 364).

(85) Choueiri TK, Pal SK, McDermott DF et al. Efficacy of cabozantinib (XL184) in patients (pts) with metastatic, refractory renal cell carcinoma (RCC). 2012 ASCO Annual Meeting. Abstract No: 4504.

(86) Rahuel J, Gay B, Erdmann D et al. Structural basis for specificity of Grb2-SH2 revealed by a novel ligand binding mode. Nat Struct Biol 1996; 3(7):586-589.

(87) 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(1):13-20.

(88) Neckers L. Hsp90 inhibitors as novel cancer chemotherapeutic agents. Trends Mol Med 2002; 8(4 Suppl):S55-S61.

(89) 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(2):342-349.

(90) Xie Q, Gao CF, Shinomiya N et al. Geldanamycins exquisitely inhibit HGF/SF-mediated tumor cell invasion. Oncogene 2005; 24(23):3697-3707.