Hereditary Papillary Renal Carcinoma Type I

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Abstract: Germline missense mutations in the tyrosine kinase domain of the hepatocyte growth factor/scatter factor (HGF/SF) receptor, c-Met, are thought to be responsible for hereditary papillary renal carcinoma (HPRC) type 1, a form of human kidney cancer. In addition to extensive linkage analysis of HPRC families localizing the HPRC type 1 gene within chromosome 7, the demonstration that individual c-Met mutations reconstituted in cultured cells display enhanced and dysregulated kinase activity, and confer cell transformation and tumorigenicity in mice, solidifies this conclusion. Our prior knowledge of HGF/SF biology and c-Met signaling enabled rapid progress in unraveling the molecular pathogenesis of HPRC type 1, and in laying the framework for the development of novel therapeutics for the treatment of this cancer. At the same time, the study of HPRC type 1 has refined our appreciation of the oncogenic potential of c-Met signaling, and challenges our current understanding of HGF/SF and c-Met function in health and disease.

Keywords: c-Met, HGF/SF, HPRC, missense mutations, renal carcinoma.

INTRODUCTION

More than thirty five thousand new cases of kidney cancer are projected to be diagnosed in the United States in 2004, the majority of which are renal cell carcinoma (RCC), which accounts for 3% of all adult malignancies. More than 12,000 deaths annually in the United States are attributed to RCC [1]. The number of RCC cases reported per year is also increasing: during the period from 1975-1995, there was an annual increase in RCC incidence of 2.3% in white men, 3.1% in white women, 3.9% in black men and 4.3 % in black women [2]. Kidney tumors are classified into 4 main types according to clinical and histological criteria. The most prevalent form, clear cell RCC, accounts for 75% of the cases, papillary renal carcinoma (PRC) accounts for 15%, chromophobe, 5% and oncocytoma, 5%. PRC is further classified into type 1 (5% of cases) and type 2 (10% of cases) based on additional clinical, histological and genetic criteria [1, 2].

In an effort to establish uniform, reliable and unambiguous criteria for classifying papillary renal neoplasms, Kovacs and coworkers stipulated that the tumor should be 75% papillary and/or tubulopapillary in architecture to be classified as PRC [3]. Delahunt and Eble refined the histopathological classification of PRC into types 1 and 2 [4]. In this organization, type 1 is generally characterized by small basophilic cells with pale cytoplasm, small oval nuclei and inconspicuous nucleoli organized in single layers in papillae and tubular structures, whereas type 2 consists of pseudo-stratified papillae composed of larger eosinophilic cells with large spherical nuclei and prominent nucleoli [4]. In addition to histological criteria, PRC has been distinguished from clear cell RCC on the basis of cytogenetic alterations. These include the gain of 2 or more of chromosomes 7, 12, 16, 17, or 20 and loss of the Y chromosome in men [5]. Alterations that are characteristic of clear cell RCC, such as loss of the short arm of chromosome 3, are not typically observed in PRC [2, 6].

Correlating clinical, histological, and cytogenetic features of PRC with linkage analysis of families with multiple affected individuals, Zbar and coworkers described an inherited form of PRC characterized by a predisposition to develop multiple, bilateral papillary renal tumors, as well as autosomal dominant transmission with frequent trisomy of chromosome 7, and suggested that it was a hereditary counterpart of the sporadic disease recognized earlier by Kovacs and colleagues [7, 8]. Through linkage analysis of an extended set of HPRC families, Schmidt and coworkers localized the HPRC gene to chromosome 7q31-34, identified missense mutations in the *MET* gene within this region that were homologous to those in other receptor tyrosine kinase proto-oncogenes mutated in human neoplasias, and proposed that gain-of-function mutations in *MET* promote tumorigenesis in HPRC type 1 [9]. Recent progress in our understanding of the pathogenesis of HPRC type 1, and particularly in the role of the *MET* gene product, the c-Met receptor tyrosine kinase, in this process, is the focus of this review.

Clinically, individuals with HPRC type 1 may develop bilateral, multifocal macroscopic and microscopic kidney lesions; macroscopic lesions tend to grow slowly and thus patients present symptoms late in life, often in their 4th, 5th or 6th decades, or die

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of other causes. Rare cases with earlier onset of the disease have been found through genetic screening of HPRC families [2]. Patients with advanced disease may present with hematuria, abdominal pain and abdominal mass; many tumors have been detected by imaging studies following positive genetic testing or incidentally by imaging studies for unrelated conditions. Computed tomography (CT) imaging studies of HPRC type 1 tumors generally show hypoenhancement following intravenous administration of a contrast agent. Because of their hypovascular nature, HPRC type 1 tumors can be mistaken for kidney cysts, which increase in incidence with age in the general population. Abdominal CT is recommended for evaluation of individuals at risk, as even relatively large papillary renal tumors may go undetected by renal ultrasound. Diagnosis is confirmed by detection of germ line mutation of the *MET* gene. Histopathologically, HPRC renal lesions display a papillary type 1 phenotype that is distinct from other sporadic papillary renal tumors [10]. While all HPRC lesions display this characteristic histotype, it is important to note that not all type 1 sporadic papillary renal carcinomas harbor somatic *MET* mutations [10].

Disease management depends on the stage and size of the tumors; HPRC type 1 tumors may become metastatic if left untreated. Large tumors are often treated with radical nephrectomy. Tumors discovered at diameters less than 3 cm are generally low grade and in these cases nephron-sparing approaches are recommended [11]. The goals of the latter approach are prevention of metastasis and preservation of renal function, because of the high likelihood of tumor recurrence, and the high mortality rate and low quality of life seen with hemodialysis or renal transplantation. Additional information regarding clinical diagnosis, staging, natural history and treatment of HPRC type 1 is available from other sources [2, 6].

c-Met FUNCTION

The *MET* gene encodes the Hepatocyte Growth Factor/Scatter Factor (HGF/SF) receptor protein tyrosine kinase, c-Met. A brief review of the HGF/SF/c-Met signal transduction pathway is provided here to put into context the role of *MET* missense mutations in the molecular pathogenesis of HPRC type 1. For further information about the biology of HGF/SF/c-Met signaling and its roles in development and homeostasis, the reader is referred to more comprehensive reviews of these topics [12-14].

HGF/SF is a pleiotropic heparin-binding protein discovered for its mitogenic activity on hepatocytes and epithelial cells, and independently discovered for its ability to stimulate cell motility (scatter) [15, 16]. HGF/SF is structurally similar to plasminogen, and undergoes proteolytic cleavage to form a biologically active disulfide linked heterodimer [16]. Several different proteases can catalyze this cleavage, including tissue- and urokinase type plasminogen activators, and coagulation factor XII. The mature HGF alpha chain is composed of an amino-terminal hairpin loop domain and four kringle domains similar to those of plasminogen, while the beta chain contains a protease-like domain which, unlike plasminogen, is not catalytically active [16]. Two naturally occurring truncated HGF/SF isoforms composed of the amino-terminal domain (N) and terminated after kringle 1 (NK1) or kringle 2 (NK2) are encoded by alternative mRNA transcripts [17]. The NK1 isoform possesses all of the basic biological activities of the full-length protein and facilitated localization of the heparin and receptor binding sites to the N and K1 domains, respectively [18-21]. Structures for the N-domain and NK1 have been obtained which have provided insights into the molecular mechanism of receptor activation and the critical role of heparan sulfate proteoglycan in that process [22-25].

HGF/SF 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 and melanocytes during embryonic development and throughout adulthood, in normal and pathological processes [13]. HGF/SF is essential for embryonic development, where it is involved in somite migration, limb bud and limb skeletal muscle formation, placenta formation [26, 27] and later in organogenesis [28], in neural development [12] and in tissue repair and regeneration [29, 30]. While the role of HGF/SF in adult renal physiology is not yet completely understood, the kidney is an important source of circulating HGF/SF in adults, and a growing body of evidence suggests that it is an endogenous renoprotective factor with potent antifibrotic activity [31].

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 *TPR* (translocated promoter region) locus on chromosome 1 fused to sequences from the *MET* locus on chromosome 7 (TPR-MET) [32]. This rearrangement has been found in patients with gastric carcinoma [33]. Isolation of the full-length *MET* proto-oncogene coding sequence revealed structural features of a membrane spanning receptor tyrosine kinase [32]. The identification of HGF/SF as the natural ligand for c-Met and the identity of SF and HGF united a collection of findings demonstrating that a single receptor transduced multiple biological activities including motility, proliferation, survival and branching morphogenesis [13]. Activation of the c-Met intrinsic tyrosine kinase (TK) activity was required for all of these activities. Consistent with its relationship with HGF/SF, c-Met is widely expressed early in development, deletion of the gene is embryonic lethal in mice, and widespread expression persists throughout adulthood [13]. Both HGF/SF and c-Met are upregulated after kidney, liver

or heart injury, suggestive of a general mechanism of protection against tissue damage, as well as one of tissue repair and regeneration [34-36].

Upon HGF/SF binding, c-Met autophosphorylation occurs on two tyrosine residues (Y1234 and Y1235) within the activation loop of the TK domain which significantly enhance kinase activity, while phosphorylation on two tyrosine residues near the carboxyl terminus of the receptor (Y1349 and Y1356) form a multifunctional docking site that recruits a collection of intracellular signal effectors containing Src homology-2 (SH2) domains and other specific receptor recognition motifs that act as adapters in transmitting signals further downstream [14, 15]. An intact multifunctional docking site is required to mediate transformation and induce a metastatic phenotype [37]. Among the adapter proteins and direct kinase substrates thus far implicated in c-Met signaling are Grb2, Gab1, phosphatidylinositol 3-kinase (PI3K), phospholipase C-γ (PLCγ), Shc, Src, Shp2, Ship1, and STAT3 [14]. Gab 1 and Grb2 are considered critical effectors and are among those which interact directly with the receptor; through these, a larger network of adaptor proteins are involved in signaling, presumably contributing to the pleiotropic biological effects elicited by HGF/SF stimulation. In particular, the direct binding of Grb2 directly to the c-Met docking site through Y1356 links the receptor to the Ras/MAPK pathway regulating cell cycle progression [14]. Gab1 is recruited to c-Met through direct binding and indirectly via Grb2; these interactions initiate branching morphogenesis in several epithelial and vascular endothelial cell types [28, 38]. Gab1 is also highly phosphorylated by the c-Met kinase, resulting in the additional recruitment of PI3K (which is also recruited to c-Met directly through its p85 subunit), contributing in turn to cell cycle progression, protection from apoptosis, as well as increased cell motility [28]. Among the many genes upregulated in response to activation of this pathway is that of the receptor itself, creating the potential for c-Met overexpression in otherwise normal target cells through persistent ligand stimulation [15]; c-Met overexpression is widely observed in cancers of epithelial origin.

HGF/SF and c-Met are implicated in a wide variety of human malignancies including colon, gastric, bladder, breast, kidney, liver, lung, head and neck, thyroid and prostate, but also sarcomas, hematological malignancies, melanoma and central nervous system (CNS) tumors [13, 39]. 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. Inappropriate c-Met signaling in disease can resemble, at least in part, developmental transitions between epithelial and mesenchymal cell types normally regulated by HGF/SF. Tumors of epithelial origin show c-Met overexpression and paracrine delivery of HGF/SF results in dysregulated signaling, whereas cells of mesenchymal origin that normally express HGF/SF often acquire c-Met expression, and several sarcomas display autocrine c-Met signaling [32]. Importantly, the c-Met pathway activates 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* [39]. 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. Hypoxia alone upregulates c-Met expression and enhances HGF/SF signaling in cultured cells and mouse tumor models [40].

MET MUTATIONS IN HPRC TYPE 1 AND PRC

Several missense mutations in *MET* have been identified in individuals with PRC, HPRC type 1, in other human cancers, as well as in cancer cell lines. In this review, missense mutations in exons will be referred to by amino acid residue, in single letter code, followed by numerical position, while amino acid changes (mutations) at the same position will be indicated after the position number in single letter code, e.g. methionine residue 1250 is M1250, and the mutation of methionine to threonine at position 1250 is denoted M1250T. Mutations in *MET* have been reported in the context of two different sequences archived in the Swissprot database (accession P08581) or GenBank (accession J02958). These sequences diverge at S755, where the GenBank sequence contains an 18 residue insert (TWW…FAS) resulting in a corresponding shift in the numbering of subsequent residues, and at Swissprot position 1191, the equivalent of GenBank 1209, where the residue is G or A, respectively. At the time of this writing, the *MET* reference sequence in LocusLink (REFSEQ accession NM_000245.2), the curated database of the National Center for Biotechnology Information (National Library of Medicine, National Institutes of Health, Bethesda, MD), is consistent with the Swissprot sequence. Tables **1** and **2** provide a compilation of all published *MET* mutations indicating their position in both numbering schemes. Throughout this review, position numbering will be consistent with the scheme used in the cited literature. To help the reader identify which numbering system is in use, GenBank numbers will be distinguished by an asterisk, e.g. M1268* (identical to Swissprot M1250). To help clarify the identity of the mutations in the context of both numbering schemes, they are depicted schematically in Figure **1**.

Schmidt and coworkers 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 [9]. Five germ line mutations and four somatic mutations were localized to the c-Met TK domain (Table **1**). Of the five

Table 1. Missense *MET* mutations in HPRC and PRC tumors and RCC cell lines.

Genbank	Swissprot	Germline/Somatic	Cancer Type /Cell lines	Exon	Domain	References
N375S	*N375S*		Cell lines[1]	2	EC	[107]
T1010I	*T992I*		Cell lines[1]	14	JM	[44]
V1110I	*V1092I*	G	HPRC	16	TK	[44, 45]
H1112Y/L	*H1094Y/L*	S	PRC	16	TK	[44]
H1112Y	*H1094Y*	G	HPRC	16	TK	[44]
H1112R	*H1094R*	G	HPRC	16	TK	[43]
H1124D	*H1106D*	S[2]	PRC	16	TK	[44]
M1149T	*M1131T*	G	HPRC	17	TK	[9]
V1206L	*V1188L*	G	HPRC	18	TK	[9]
A1209G	*A1191G*		Cell lines[1]	18	TK	[107]
L1213V	*L1195V*	S	PRC	18	TK	[9]
V1238I	*V1220I*	G	HPRC	19	TK	[9, 44]
D1246N	*D1228N*	G	HPRC	19	TK	[9]
D1246H	*D1228H*	S	PRC	19	TK	[9]
Y1248D	*Y1230D*	G	HPRC	19	TK	[44]
Y1248C	*Y1230C*	G	HPRC	19	TK	[9]
Y1248C	*Y1230C*	S	PRC	19	TK	[44]
Y1248H	*Y1230H*	S	PRC	19	TK	[9]
M1268T	*M1250T*	S	PRC	19	TK	[9]
M1268T	*M1250T*	G	HPRC	19	TK	[44]
V1290L	*V1272L*		Cell lines[1]	19	TK	[107]

Mutations have been reported in the context of two different sequences archived in the Swissprot database (accession P08581) or Genbank (accession J02958). The numbering used in the original citation (rightmost column) is shown in normal type, while the corresponding position in the alternative numbering scheme is shown in italics. Where multiple amino acid substitutions have been found at the same position, they are listed consecutively after the position number. G indicates germline mutations, S indicates somatic mutations. TK indicates tyrosine kinase domain, JM indicates juxtamembrane domain, and EC indicates extracellular domain. [1]Human PRC cell lines ACHN and VMRC-RCW; whether these mutations were somatic or germline was not determined. The change T1010I* has been reported both as a mutation and possible polymorphism. [2]Somatic by presumption; no normal tissue was available for comparison.

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. 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. The absence of mutations at these positions in a large panel of normal individuals indicated that these were not likely to be polymorphisms [9].

The biochemical and biological impact of these *MET* mutants were investigated in NIH3T3 cell transfectants [41]. Mutant c-Met receptors displayed increased levels of tyrosine autophosphorylation relative to wild type (WT) receptors, as well as greater TK activity towards an exogenous substrate. 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 c-Met in the same context. The somatic mutations were generally more active in these assays than the germ line mutations. Subsequently the same group showed that mutant receptors showed increased cell motility relative to WT in the absence of HGF, as well as increased intracellular activation of the Ras-Raf-MEK-ERK signaling pathway [42]. Finally, these investigators demonstrated that transgenic mice harboring the PRC mutant c-Met constructs under the control of a metallothionein promoter developed metastatic mammary carcinoma, solidifying the conclusion that these *MET* mutations were oncogenic [42].

A study of two large North American HPRC families resulted in the identification of a novel germ line mutation in exon 16 of both (H1112R*) [43]. This

Table 2. *MET m*utations found in tumors and cancer cell lines.

Genbank	Swissprot	Germline/ Somatic	Cancer Types /Cell line	Exon	Domain	References
E168D	*E168D*	S	SCLC	2	EC[1]	[66]
P791L	*P773L*	G	familial gastric cancer	10	EC	[59]
R988C	*R970C*	S	SCLC cell lines[2]	14	JM	[66]
P1009S	*P991S*	G	primary gastric cancer	14	JM	[58]
T1010I	*T992I*	S	SCLC breast cancer lung cancer cell line3	14	JM	[58, 66]
H1112R	*H1094R*	S	cell line4	16	TK	[63]
N1118Y	*N1100Y*	S	cell line4	16	TK	[63]
G1137V	*G1119V*	S	glioma	17	TK	[108]
T1191I	*T1173I*	S	childhood hepatocellular carcinoma	17	TK	[60]
A1209G	*A1191G*	S	ovarian carcinoma	18	TK	[109]
Y1248C	*Y1230C*	S	cell line4	19	TK	[61, 63]
Y1253D	*Y1235D*	S	cell line4	19	TK	[61, 63]
K1262R	*K1244R*	S	childhood hepatocellular carcinoma	19	TK	[60]
M1268I	*M1250I*	S	childhood hepatocellular carcinoma	19	TK	[60]
IVS13 [52-53] ins CT		Insertional mutations	SCLC	13[5]	pre-JM	[66]
Ex 10		Alternative splicing	SCLC cell lines[4]	10[6]	EC	[66]

Mutations are reported as described for Table **1**. [1]Mutation falls within the Sema domain, which contains the putative ligand binding site. [2]Cell lines NCI-H69 and H249; [3]large cell lung cancer cell line Hop-92; [4]SCLC cell line 128. [5]Mutation consists of an insertion into intron 13. [6]Entirety of exon 10 deleted.

mutation significantly enhanced focus formation when ectopically expressed in NIH3T3 cells, suggesting that it also was oncogenic. The H1112R* mutation is located in the amino-terminus of the TK domain close to the glycine rich region involved in ATP binding and in stabilizing the amino- and carboxyl-terminal lobes of the TK domain. Using an extended panel of 79 sporadic PRC samples, 5 additional missense mutations were detected in *MET*, 3 of which were also found to be germline mutations through comparison with matched normal samples, despite the absence of family history of disease in those cases (Table **1**) [44]. Missense mutations reported in earlier studies of HPRC families were also found in that panel of tumor samples. One of these mutations, V1110I*, is also located within the highly conserved glycine rich ATP binding region of the tyrosine kinase domain, and was identified independently by another group studying an Italian HPRC family [45]. This mutation is found in the homologous codon (V157I) of chicken *c-erbB*, and triggers the sarcomagenic potential of the *v-erbB* oncogene [46-48], suggestive of an oncogenic role in HPRC type 1.

While prior studies focused on exons 16-19 of the TK domain, the analysis of the extended panel of tumor samples by Schmidt and coworkers 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 [44]. The results 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 [49]. 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 *MET* mutation contributes to errors in chromosomal replication during cell division, and that having two copies of the mutant allele confers a proliferative advantage leading to clonal expansion of the affected tumor cells [50]. While this potential mechanism of selective overexpression of mutant c-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. Whether the potentially increased dose of *MET* and/or *HGF* genes, both located on chromosome 7, confers a proliferative advantage in the absence of mutation is an attractive hypothesis that warrants further investigation.

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751  KSFIS                           GGSTITG VGKNLNSVSV PRMVINVHEA
751  KSFISTWWKE PLNIVSFLFC FASGGSTITG VGKNLNSVSV PRMVINVHEA

783  GRNFTVACQH RSNSEIICCT TPSLQQLNLQ LPLKTKAFFM LDGILSKYFD
801  GRNFTVACQH RSNSEIICCT TPSLQQLNLQ LPLKTKAFFM LDGILSKYFD

833  LIYVHNPVFK PFEKPVMISM GNENVLEIKG NDIDPEAVKG EVLKVGNKSC
851  LIYVHNPVFK PFEKPVMISM GNENVLEIKG NDIDPEAVKG EVLKVGNKSC

883  ENIHLHSEAV LCTVPNDLLK LNSELNIEWK QAISSTVLGK VIVQPDQNFT
901  ENIHLHSEAV LCTVPNDLLK LNSELNIEWK QAISSTVLGK VIVQPDQNFT

933  GLIAGVVSIS TALLLLGFF LWLKKRKQIK DLGSELVRYD ARVHTPHLDR
951  GLIAGVVSIS TALLLLGFF LWLKKRKQIK DLGSELVRYD ARVHTPHLDR

983  LVSARSVSPT TEMVSNESVD YRATFPEDQF PNSSQNGSCR QVQYPLTDMS
1001 LVSARSVSPT TEMVSNESVD YRATFPEDQF PNSSQNGSCR QVQYPLTDMS

1033 PILTSGDSDI SSPLLQNTVH IDLSALNPEL VQAVQHVVIG PSSLIVHFNE
1051 PILTSGDSDI SSPLLQNTVH IDLSALNPEL VQAVQHVVIG PSSLIVHFNE

1083 VIGRGHFGCV YHGTLLDNDG KKIHCAVKSL NRITDIGEVS QFLTEGIIMK
1101 VIGRGHFGCV YHGTLLDNDG KKIHCAVKSL NRITDIGEVS QFLTEGIIMK

1133 DFSHPNVLSL LGICLRSEGS PLVVLPYMKH GDLRNFIRNE THNPTVKDLI
1151 DFSHPNVLSL LGICLRSEGS PLVVLPYMKH GDLRNFIRNE THNPTVKDLI

1183 GFGLQVAKGM KYLASKKFVH RDLAARNCML DEKFTVKVAD FGLARDMYDK
1201 GFGLQVAKAM KYLASKKFVH RDLAARNCML DEKFTVKVAD FGLARDMYDK

1233 EYYSVHNKTG AKLPVKWMAL ESLQTQKFTT KSDVWSFGVV LWELMTRGAP
1251 EYYSVHNKTG AKLPVKWMAL ESLQTQKFTT KSDVWSFGVV LWELMTRGAP

1283 PYPDVNTFDI TVYLLQGRRL LQPEYCPDPL YEVMLKCWHP KAEMRPSFSE
1301 PYPDVNTFDI TVYLLQGRRL LQPEYCPDPL YEVMLKCWHP KAEMRPSFSE

1333 LVSRISAIFS TFIGEHYVHV NATYVNVKCV APYPSLLSSE DNADDEVDTR
1351 LVSRISAIFS TFIGEHYVHV NATYVNVKCV APYPSLLSSE DNADDEVDTR

1383 PASFWETS
1401 PASFWETS
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Figure. 1. *MET* mutations in human cancers.

Partial sequence of the c-Met receptor protein encompassing reported missense mutations is shown. Mutations have been reported in the context of two different sequences archived in the Swissprot Database (shown in red; accession P08581) or GenBank (shown in black; accession J02958). The sequences diverge at S755, where the GenBank sequence contains an 18 residue insert (TWW…FAS; shown in blue) resulting in a shift in codon position numbering, and at position 1191/1209* as indicated. The latter difference was also reported as a missense mutation (A1209G*; Tanyi *et al*., Pathol Oncol Res, 1999). A reference sequence obtained from the curated LocusLink Database (National Center for Biotechnology Information, National Institutes of Health, USA; REFSEQ accession NM_000245.2) lists G at this position, so we do not depict this difference as a missense mutation here. Boxes denote positions of Met mutations detailed in Tables **1** and **2**; blue boxes indicate germline mutations, yellow boxes indicate somatic mutations and green boxes indicate that the mutation was reported as both germline and somatic. The gray box marks transmembrane domain, the TK domain is underlined, and the glycine-rich ATP binding region within the kinase domain is indicated by an unshaded box. Mutations found in HPRC type 1 and PRC are indicated by an asterisk beneath the codon position.

Several studies have addressed in detail the mechanisms by which PRC-associated c-Met mutations act at the cellular and molecular levels. Bardelli and colleagues showed that the M1250T mutation changed substrate preference *in vitro,* using a panel of peptides differentially

phosphorylated by epidermal growth factor receptor (EGFR), Src, or Abl; M1250T conferred the acquisition of an Abl-like pattern of substrate preference, similar to that displayed by the homologous *RET* mutation characteristic of MEN 2B [51]. In the context of NIH3T3 cells, the mutations Y1230H, D1228H/N and M1250T showed constitutive association with the key intracellular effector Gab1. Similar to signaling by WT c-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 [51]. The results revealed that oncogenicity is mediated by many of the receptor-proximal intracellular effectors involved in WT c-Met signaling, and that interruption of key receptor-effector interactions at the carboxyl-terminal docking sites might be a viable strategy for blocking mutant c-Met signaling [51].

Building upon prior studies that demonstrated different NIH3T3 transforming abilities by different PRC-associated mutations, Giordano and coworkers hypothesized that different mutations may contribute to disease pathogenesis through distinct molecular pathways downstream of c-Met [52]. When ectopically expressed in NIH3T3 cells or the murine liver oval cell line MLP 29, the *MET* PRC mutants studied fell into two functional groups: M1250T and D1228H possessed enhanced receptor kinase activity, stimulated increased Ras pathway activation and transformed recipient cells in focus formation assays. Mutations L1195V and Y1230C, in contrast, displayed lower kinase activity, Ras pathway activation and focus forming ability, but were more effective in PI3K pathway activation, protecting recipient cells from apoptosis, sustaining soft agar colony formation and promoting invasion in Matrigel [52]. All of these effects were enhanced upon addition of HGF/SF [52]. How these findings correlate with different features among PRC tumors harboring different mutations is not yet clear, but they suggest that signal divergence downstream of mutant c-Met forms should be considered in the development of targeted therapeutics for HPRC type 1.

The role of ligand binding in the oncogenic potential of PRC-associated c-Met mutations was extensively investigated by Michieli and coworkers using cultured cell systems [53]. NIH3T3 cells coexpressing mutant c-Met receptors and HGF showed generally greater focus forming activity than in the absence of HGF/SF expression (particularly L1195V, Y1230H, and M1250T). It is worth noting here that the mouse fibroblast cell line NIH3T3 has been extensively used for the analysis of c-Met function, probably because it lacks significant endogenous c-Met expression, is readily transfected, and amenable to a variety of assays correlated with tumorigenicity in mice, such as focus formation in monolayer culture. However, NIH3T3 cells express and secrete HGF, and some crossreactivity between murine HGF and human c-Met has been observed. This provided an impetus to reconstitute c-Met mutants in epithelial cells, which typically do not express HGF. Indeed, c-Met mutants reconstituted in MDCK epithelial cells required exogenously added ligand for colony formation in soft agar [53]. c-Met mutations reconstituted in truncated receptor constructs lacking most of the extracellular domain failed to induce focus formation, and M1250T reconstituted in this context was transforming only upon addition of a receptor-ligating monoclonal antibody [53]. Finally, soft agar colony formation by NIH3T3 cells bearing c-Met M1250T could be blocked by coexpression of a soluble c-Met extracellular domain (decoy Met), an uncleavable form of HGF/SF, or the HGF/SF competitive antagonist NK4 [53]. Together these results revealed that ligand binding may contribute significantly to oncogenesis associated with PRC *MET* mutations [53]. These investigators further speculated that ligand dependence may explain why patients with germline *MET* mutations exhibit only kidney cancer: the kidney is an abundant source of HGF/SF, as well as urokinase, an important activator of secreted, immature HGF/SF [53]. The long term combination of ligand, ligand activator, heparan-sulfate glycosaminoglycan, and highly responsive target cells may render these otherwise benign receptor mutations “regionally” oncogenic.

To predict how PRC c-Met mutations might alter catalytic function, Miller and colleagues aligned the TK domain of c-Met with that of the insulin receptor (IR), the most closely related receptor for which a crystal structure had been obtained [54]. Using computer modeling methods, the aligned c-Met sequence was superimposed on the IR crystal structure coordinates, generating 3D models of WT and mutant c-Met molecules in basal and catalytically active conformations. The results predicted that certain HPRC type 1 mutations could disrupt the normal mechanism of TK autoinhibition, thereby stabilizing the active form of the receptor [54]. In the unphosphorylated form of the WT receptor, residues in the A-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. Notably, the HPRC type 1 mutation M1268T* was predicted to stabilize the active conformation of the A-loop, thereby increasing the interaction of the catalytic pocket with the carboxyl-terminal phosphorylation/docking sites and other peptide substrates [54]. Phosphorylation of Y1248* in WT c-Met is thought to stabilize the open TK conformation by facilitating ionic and/or hydrophilic interactions by this otherwise buried residue, a state that is highly reversible through the action of closely associated protein tyrosine phosphatases. Mutation of Y1248* to the more hydrophilic residues C, D, or H would be predicted to more permanently stabilize the active TK conformation by rendering the site resistant to phosphatase action. Other mutants appeared to facilitate flexibility in critical points

among molecular modules, enabling subdomain movements that might change substrate binding ability [54]. Overall, these findings predicted that mutant c-Met forms might be more easily activated than WT c-Met, and more likely to remain active once activated, but did not provide a mechanism that clearly obviated the need for an initiator of kinase activation, such as ligand binding, activation by receptor cross-talk, or other environmental cue.

In a study that functionally complements the work of Miller *et al.*, Chiara and colleagues compared the autophosphorylation events in WT and mutant c-Met receptors expressed in cultured cells using phosphorylation-site specific antibodies, and proposed that mutant receptors possessed a lower threshold for kinase activation [55]. Earlier studies established that WT c-Met triggered by HGF/SF binding undergoes 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 [56, 57]. Unlike WT c-Met, Chiara *et al.* found that D1228H/N and M1250T c-Met mutants did not undergo Y1234 phosphorylation, and were not catalytically impaired by F substitutions at that site. Thus these mutants were not constitutively active, but mutation overcame the normal requirement for a second phosphorylation step leading to kinase activation [55]. Chiara and colleagues also speculated that an apparent requirement for HGF is consistent with the restriction of HPRC type 1 to the kidneys of individuals carrying germline *MET* mutations, where both HGF/SF and urokinase capable of HGF/SF activation are relatively abundant [55].

MET MUTATIONS IN OTHER HUMAN TUMORS

Beyond PRC, the HGF/SF-c-Met pathway has been implicated in a wide range of human cancers primarily through inappropriate or abnormally high expression not associated with receptor mutation. However, *MET* mutations have been found in some non-PRC cancers (Table **2**). Lee and colleagues screened 85 cases of primary gastric cancer for *MET* mutations and identified a novel germline missense *MET* mutation, P1009S*, in the juxtamembrane (JM) domain [58]. When expressed in NIH3T3 cells, c-Met P1009S* was not constitutively phosphorylated, but displayed increased and persistent HGF/SF-stimulated autophosphorylation, as well as enhanced tumorigenicity in nude mice, relative to WT c-Met [58]. Consistent with these observations, the JM domain contains a protein kinase C phosphorylation site, a PEST sequence, and a recognition site for c-Cbl, all three of which may negatively regulate normal c-Met signaling. Also noted in a breast cancer sample was the change T1010I*, which had been observed earlier in PRC and large cell lung cancer cell lines as well as in the germline of an individual with a family history of PRC, but had been considered a polymorphism because it did not segregate with disease in that family. The T1010I* mutation did not enhance c-Met phosphorylation in NIH3T3 cells but was weakly tumorigenic in nude mice [58].

In a screen of 21 Korean families affected with diffuse familial gastric cancer, Kim and coworkers found one germ line missense mutation, P791L*, in exon 10 of the *MET* extracellular domain [59]. The low frequency of this mutation and the absence of data supporting an oncogenic mechanism render its overall functional significance to disease onset and progression unclear at this time. Park and colleagues screened a panel of 75 primary liver carcinoma samples for *MET* mutations in exons 15-19 of the TK domain [60]. They found three somatic missense mutations, T1191I*, M1268I*, and K1262R*, among 10 childhood hepatocellular carcinoma (HCC) cases, but no mutations in 16 adult HCC samples, 21 cholangiocarcinomas, or 28 hepatoblastomas [60]. The M1268I* mutation occurred at the same site as somatic and germline PRC mutations, while the other two were novel mutations in the TK domain with unknown functional impact. The absence of mutations in 16 adult HCC cases contrasted strikingly with the relatively high frequency (3/10) of mutations in childhood HCC, leading Park *et al.* to speculate that *MET* mutation may contribute to the earlier onset of the childhood disease [60].

The hypothesis that aberrant activation of the HGF/SF-c-Met pathway during tumor progression promotes metastasis is extensively supported by studies in model systems [39]. Consistent with this hypothesis, Di Renzo and colleagues found two activating somatic *MET* mutations, Y1230C and Y1235D, well-represented in lymph node metastasis of head and neck squamous cell carcinoma (HNSCC), but absent from primary tumors [61]. Of these, the novel Y1235D mutation showed enhanced kinase activity and promoted colony formation in soft agar when reconstituted in cultured cells [61]. Y1234 and Y1235 are the two essential phosphorylation sites involved in c-Met TK activation, and several studies suggest that Y1235D may lower the threshold for kinase activation, as well as render the activation loop resistant to closure through dephosphorylation, providing hypersensitive and persistent signaling [54, 55, 57, 61, 62]. The observations of Di Renzo *et al*. strongly suggest that activating *MET* mutations acquired in a subpopulation of primary HNSCC tumor cells promotes their clonal expansion and lymph node metastasis [61]. In a follow-up study by the same group, Lorenzato *et al.* reported the Y1253D* (identical to Y1235D) mutation in a lung metastasis of a colorectal carcinoma, and identified a novel mutation, N1118Y*, in a lung metastasis in a patient with HNSCC [63]. The latter mutation was just carboxyl-terminal to the glycine rich region involved in ATP binding and the H1112Y/L/R* mutations found in PRC patients. Consistent with metastatic potential, this mutation promoted increased motility and matrix

invasion when reconstituted in cultured cells [63]. Together, the two studies encompassed the analysis of 153 sporadic human cancer samples and 25 cancer cell lines, uncovering little evidence of *MET* mutation in primary tumors, but mutations in 10 of 46 lymph node metastases (from 4 patients) and 2 of 14 pulmonary metastases [61, 63]. Thus for tumors other than PRC, *MET* TK domain mutations have been observed predominantly in metastases rather than in primary lesions [63].

Prior studies have shown that the c-Met pathway is functional and relevant in small cell lung cancer [64, 65]. Ma and colleagues performed mutational analysis of the entire *MET* gene in 10 SCLC cell lines and 32 SCLC samples with paired normal tissues, and identified novel somatic missense mutations and alternatively spliced mRNA transcripts [66]. Mutations R988C*, found in NCI-H69 and H249 cell lines, and T1010I*, found in a SCLC tumor sample, were both located in the JM domain [66]. Another mutation, E168D*, identified in a tumor sample, was located in the putative ligand binding domain [66]. When reconstituted in cultured cells, the JM mutations abrogated cytokine dependence, increased motility and promoted colony formation in soft agar, suggestive of oncogenicity [66]. While the precise mechanism(s) by which JM mutations may enhance c-Met signaling are not yet defined, they include the loss of negative regulatory events such as c-Cbl interaction, and/or constitutive association with positive downstream intracellular effectors, many of which have been implicated in human cancers both independently of, and in the context of, c-Met signaling.

c-Met ASSOCIATED MOLECULES IMPLICATED IN CANCER

An increasing amount of work has revealed regulation of c-Met TK activation through other receptors able to form multiprotein complexes on the cell surface. The ability of ligand activated EGR receptor to transphosphorylate c-Met and initiate its intracellular signaling pathway has been demonstrated in cultured cells and implicated in oncogenesis [67]. Semaphorin 4D, a soluble factor best characterized in the regulation of axonal guidance, binding to its cell surface receptor plexin B1, also stimulates c-Met TK activity independent of HGF/SF, and requires this interaction to elicit an invasive growth response [68]. Cell adhesion and spreading itself has been shown to activate c-Met [69, 70], and c-Met signaling in anchorage-dependent cell types is very likely to cooperate with extracellular cues from matrix components and intercellular contacts [71]. Thus signaling from these sources may contribute to the pathologies associated with c-Met mutations. While mutations in c-Met-associated molecules and/or effectors have not yet been found in PRC, gastric cancer, or SCLC, changes in effector expression level, intracellular localization or turnover have been implicated in human cancers and may contribute to oncogenesis associated with *MET* mutations.

At the cell surface, c-Met has been shown to interact with $\alpha6\beta4$ integrin, and to induce tyrosine phosphorylation of the $\beta4$ subunit resulting in the recruitment of other intracellular effectors such as Shc and PI3K, thereby enhancing HGF-stimulated invasiveness and transforming activity in cultured cells [72]. This integrin complex is normally involved in the formation of epithelial junctions called hemidesmosomes [73], but by binding to the actin cytoskeleton in pathological situations, participates directly in cell migration [74]. Whether $\alpha6\beta4$-c-Met interaction is required for c-Met-mediated transformation remains controversial [75]. c-Met is also physically associated with CD44, a widely expressed class 1 transmembrane glycoprotein produced in a variety of isoforms and sufficient to confer metastatic potential *in vitro* [76, 77]. The role of CD44 in this context may be related to its abundance on the surfaces of transformed cells, which also overexpress c-Met [78, 79] and the presentation of HGF to c-Met by CD44 heparan sulfated side chains [80], promoting receptor activation. In addition, the intracellular domain of CD44 interacts with cytoskeletal proteins (e.g. actin) and signal transducers (e.g. Src and MAPK), bringing these targets in close proximity of c-Met to form a cytoplasmic supramolecular complex [81].

Acting as an intercellular junction component and signaling molecule in several pathways including downstream of c-Met, β-catenin has been implicated in human cancers though at least four mechanisms: mutations of β-catenin, adenomatous polyposis coli (APC) or axin genes, and activation of Wnt signaling [82-84]. Normally cytoplasmic β-catenin is quickly targeted for degradation via interactions with APC, axin, and other proteins [82]. In the canonical Wnt pathway, Wnt binding at the cell surface destabilizes the APC/axin/β-catenin complex leading to the accumulation of cytoplasmic β-catenin, interaction with transcription factors such as Tcf4 and subsequent transcription of a variety of target genes involved in cell cycle progression, matrix remodeling, cell polarity and morphogenic changes [82, 85]. Defective APC genes found in families with the genetic cancer syndrome familial adenomatous polyposis (FAP) fail to target β-catenin for degradation resulting in constitutive Tcf4 activity; inactive mutant APC alleles are also found in most sporadic colorectal cancers [86]. Somatic mutations in β-catenin are widespread in human tumors, including melanoma, liver, colon, prostate, ovarian, and endometrial cancers [87]. Interestingly, ectopic expression of *MET* PRC/HPRC mutant M1268T* was associated with cytoplasmic accumulation of β-catenin, constitutive activation of the transcription factor Tcf-4, and Tcf-dependent accumulation of c-myc and cyclin D1 proteins, implicating β-catenin in oncogenesis via the M1268T* mutation [88]. The availability of small-molecule antagonists of the oncogenic Tcf/β-catenin complex should allow further

analysis of the potential role of β-catenin signaling downstream of MET mutations [89].

In normal cells, attenuation of HGF/SF-c-Met signaling is though to occur through several mechanisms, including ligand-stimulated receptor ubiquitination and subsequent proteosomal degradation [90]. c-Cbl, an E3 ubiquitin-protein ligase, negatively regulates signaling downstream of several receptor TKs including those for EGF, platelet-derived growth factor, colony stimulated factor-1, and as recently demonstrated, HGF/SF [91]. Preschard and coworkers identified Y1003 in the c-Met JM domain as critical for c-Cbl/c-Met interaction; it is noteworthy that *TPR-MET* does not contain Y1003 and does not interact with c-Cbl, and that *TPR-MET* is no longer transforming when the JM region is inserted [92]. Recombinantly engineered mutation of Y1003 to F resulted in the loss of c-Met polyubiquitination and the acquisition of oncogenic properties by transfected cells [91]. The homologous position of Y1003 is conserved in the c-Met family member avian c-Sea, but missing from its retroviral oncogenic counterpart *env-sea*. A putative c-Cbl site is also deleted in *v-fms*, the oncogenic variant of the CSF-1 receptor, and in several *TrkC* oncogenic variants generated by chromosomal rearrangements in acute myeloid leukemia and congenital fibrosarcoma, leading Peschard and colleagues to propose that loss of this regulatory arm may be an important contribution to oncogenicity of receptor TKs generally [91].

INSIGHTS INTO THERAPEUTIC DEVELOPMENT

In addition to improving our understanding of disease pathogenesis, the study of oncogenic mechanisms associated with *MET* mutation has provided constructive insights into strategies for therapeutic development. Certainly further analysis of these mutations in model systems, such as the development of mouse models with inducible and reversible point mutations, will strengthen our understanding of the earliest oncogenic events, as has been suggested in general for the study of oncogenic kinase signaling [93]. In parallel, the continued study of affected families will help uncover complexities inherent in the biology of humans that affect the rate of disease progression, its severity and metastatic spread. Our present understanding of oncogenesis mediated by *MET* mutation supports at least three avenues of therapeutic development: direct inhibition of TK catalytic activity, antagonism of ligand/receptor interaction, and inhibition of receptor/effector interactions. In addition, combinations of conventional therapies or conventional and targeted therapies may offer promise for specific cancers. For example, Aebersold and coworkers found that the c-Met Y1253D* mutation in patients with squamous cell carcinoma of the oropharynx correlated with a significantly lower probability of progression-free survival following radical radiotherapy, suggesting that this *MET* mutation may interfere with tumor radioresponsiveness, and that targeting c-Met signaling might afford radiosensitization for HNSCC [94].

Much evidence indicates that *MET* TK mutations enhance kinase activity, suggesting that selective TK inhibition is a viable therapeutic strategy for the treatment of PRC and HPRC type 1. In general, the importance of receptor and non-receptor TKs in cancer onset and progression has stimulated mechanism- and structure-based drug design approaches for the development of potent and selective therapeutics targeting these domains, particularly the region encompassing the ATP binding site and the activation loop. Morotti and colleagues demonstrated that one such kinase inhibitor, K252a, could inhibit c-Met autophosphorylation, MAPK activation and Akt activation thereby preventing HGF-induced cell motility and proliferation, and reverting the highly transforming phenotype of the *TPR-MET* oncogene [95]. Pretreatment of *TPR-MET* transformed fibroblasts or GTL-16 gastric carcinoma cells (which overexpress WT c-Met) with K252a blocked their ability to form lung metastases in mice [95]. K252a is a staurosporine-like alkaloid and potent (nM) antagonist of ATP binding by *Trk* family kinases [96], and while these results reveal its relatively low selectivity among c-Met and Trk kinases, they strongly suggest that TK inhibition of PRC c-Met mutants is a viable avenue for therapeutic development. Moreover, K252a appeared to have greater potency on c-Met M1268T* [95], an effect also displayed by the EGFR-directed TK inhibitor Iressa (gefitinib, ZD1839) on mutated EGFR found in recent clinical trials for non-small cell lung carcinoma [97], and a fortuitous finding when considering the possible toxicity arising from c-Met pathway blockade in patients with PRC and other cancers [95].

Recently, more selective synthetic inhibitors of ATP binding by the c-Met kinase, effective in the 10 nM concentration range in cultured cells, have been developed and tested in various model systems [98-100]. Of these, the novel pyrrole indolinone compound SU11274 displayed a minimum of 50-fold selectivity for c-Met relative to several related tyrosine kinases, and blocked *TPR-MET*-mediated transformation of the mouse myeloid BaF3 cell line, leading to both cell cycle arrest and apoptosis [99]. Further analysis of SU11274 using NIH3T3 cells expressing the *MET* mutants M1268T*, H1112Y*, L1213V* and Y1248H*, including kinase activity, intracellular effector activation, morphological transformation, cell cycling and motility assays revealed interesting differences in the susceptibility of the various mutants to this compound [100]. While the mutants M1268T* and H1112Y* were potently inhibited by this compound, L1213V* and Y1248H* were largely resistant [100]. These results reinforce the notion that these mutations affect different facets of TK activation and that genetic screening of

PRC patients may be important in predicting the therapeutic value of c-Met TK inihbitors. While the reasons for these differences in susceptibility are unclear at present, future crystallographic studies of the c-Met TK domain in the presence of SU11274 and other inhibitors will probably resolve this question and accelerate the development of c-Met inhibitors generally.

Antagonism of ligand binding is another potential therapeutic strategy for HPRC type 1 and PRC, as well as other malignancies where c-Met is not mutated but active. Indeed, several lines of evidence support a role for HGF/SF in promoting oncogenesis by c-Met mutations such as L1195V, Y1230H, and M1250T, including the inability of mutant receptors to transform epithelial cells, where HGF/SF is not expressed, restoration of this property upon ligand addition, and the ability of ligand antagonists, such as NK4, to block cell transformation [52, 53, 55]. As noted above, the prevalence of trisomy 7 in sporadic PRC in the absence of *MET* mutation may occur through a selection for increased dose of the *HGF* gene as well as *MET*, since both are located on chromosome 7. Finally, the increased risk for polycystic kidney disease and RCC, including PRC, in individuals receiving long term hemodialysis therapy, where significantly elevated levels of circulating HGF/SF have been reported, further implicates HGF/SF in the pathogenesis of these renal cancers [101].

The requirement of the carboxyl-terminal docking site for mutant c-Met transforming activity in cultured cells, as well as enhanced association of Gab1 and activation of the Ras/ERK and PI3K pathways, suggests that many of the effectors of WT c-Met signaling also promote oncogenesis by c-Met mutants [51, 52]. These findings, coupled with other studies that demonstrate the importance of receptor-proximal effectors such as β-catenin, Grb2, Shc and STAT3 in models of WT c-Met-mediated cell transformation [14, 15, 39], suggest that targeting one or more of these interactions could disrupt oncogenesis driven by *MET* mutation. One such approach targets Src homology 2 (SH2) domain binding, the means by which most effectors interact with the c-Met carboxyl-terminal docking site. SH2 domains directly recognize phosphotyrosine (pY), with additional secondary binding interactions within two or three amino acids C-proximal to the pY residue introducing differential affinity toward SH2 domain subfamilies [102]. These and other observations have led to the development of potent, low molecular weight, synthetic inhibitors of specific SH2 domain interactions [103, 104]. For example, inhibitors of the Grb2 SH2 domain potently block HGF/SF-stimulated cell motility, matrix invasion and branching morphogenesis in epithelial and hematopoietic as well as HGF/SF-, basic fibroblast growth factor-, and vascular endothelial cell growth factor-stimulated angiogenesis [105, 106]. Targeting key signaling interactions downstream of several growth factor receptors implicated in tumor progression, in both tumor and vascular cells, could potentially inhibit tumor growth, invasion and metastasis as well as the recruitment of new blood vessels needed to sustain these processes. Insights gained from the refinement of Grb2 SH2 domain binding antagonists should also aid in the development of SH2 domain antagonists selective for other critical c-Met effectors, such as STAT3, Shc and PI3K, with potential application to PRC and other cancers driven by TK signaling.

SUMMARY

MET mutations occur in a limited subset of cancers where the HGF/SF-c-Met signaling pathway is thought to contribute significantly to tumor progression and metastasis. Somatic and/or germline *MET* mutations have been found in HPRC type 1, PRC, HNSCC, SCLC, HCC, gastric cancer, ovarian cancer and glioma. Among those cancers where *MET* mutations have been found, a role for oncogenesis has been most clearly established in HPRC type 1. The germline missense *MET* mutations found in HPRC type 1 localize predominantly to the TK domain. Some of these mutations are homologous in position and/or substitution to mutations found in other TK receptors and associated with other human cancers or neoplastic diseases. The TK mutations found in HPRC type 1 appear to lower the threshold for receptor activation, stabilize the active conformation of the kinase, and in some cases render it less susceptible to inactivation by phosphatases. In other cancers, such as SCLC, the majority of mutations occur in the JM domain, where sites which mediate polyubiquitination and receptor degradation negatively regulate c-Met function. The significance of the apparent association of different *MET* mutations with distinct types of cancer is not yet fully understood. Trisomy of chromosome 7, where both *MET* and *HGF* genes are located, occurs frequently in PRC as well as in HPRC type 1, but its contribution to disease progression also remains to be defined. Ongoing investigations continue to address these and other important questions regarding the pathogenesis of HPRC type 1, such the cellular origin of renal tumors, the relationship of the *MET* TK mutations to the distinct papillary tumor architecture of HPRC type 1, and the molecular events predisposing HPRC type 1 tumors to metastasis.

Our basic understanding of the HGF/SF-c-Met signaling pathway, together with extensive analyses of tumor-associated mutant c-Met forms in model systems, suggest at least three general strategies of targeting the pathway for therapeutic development: blockade of receptor activation, inhibition of TK activity, and disruption of receptor-effector interactions. Of these, recent success in the treatment of other cancers, such as chronic myeloid leukemia, has proven in principle that inhibition of TK activity can be safe and effective. The remaining strategies, not far behind in development, also offer

promise for effective treatment. The HPRC type 1 patient population are the most likely to benefit from drugs that effectively target the HGF/SF-c-Met signaling pathway, and although they represent only a fraction of RCC cases, information gained from the treatment of these patients will be relevant to other cancers where c-Met signaling is likely to contribute to tumor progression and metastasis, such as HNSCC, SCLC, and several others. The HPRC type 1 patient population also provides an opportunity for cancer prevention trials, as individuals with germline MET mutations and family history of RCC are clearly at high risk to develop tumors. Our knowledge of the oncogenic molecular pathways, combined with a better understanding of the role of HGF/SF in adult homeostasis, tissue repair and regeneration, will aid in the development of efficacious targeted therapies with safety profiles consistent with long term administration. At the same time, ancillary biological studies should help identify surrogate markers predictive of disease stabilization, progression, and metastasis.

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