Review Article

MET in human cancer: germline and somatic mutations

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Abstract: Since the initial discovery of missense *MET* mutations in hereditary papillary renal carcinoma (HPRC), activating *MET* mutations have been identified in a diverse range of human cancers. *MET* mutations have been identified in several functional domains including the kinase, juxtamembrane, and Sema domains. Studies of these mutations have been invaluable for our understanding of the tumor initiating activity of *MET*, receptor tyrosine kinase (RTK) recycling and regulation, and mechanisms of resistance to kinase inhibition. These studies also demonstrate that mutationally activated MET plays a significant role in a wide range of cancers and RTKs can promote tumor progression through diverse mechanisms. This review will cover the various *MET* mutations that have been identified, their mechanism of action, and the significant role that mutationally-activated *MET* plays in tumor initiation, progression, and therapeutic resistance.

Keywords: Receptor tyrosine kinase (RTK); MET; missense mutation; kinase domain; receptor recycling

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Deregulated MET signaling occurs in cancer through several mechanisms including overexpression, amplification, autocrine signaling, and mutational activation. In this chapter, we focus on the breadth of *MET* activating mutations that have been discovered and their tumorigenic consequences. Studies of these *MET* mutations have been invaluable for our understanding of the tumor initiating activity of *MET*, receptor tyrosine kinase (RTK) recycling and regulation, and mechanisms of resistance to kinase inhibition. In addition, we will discuss the various tumor types where *MET* mutations have been found and what these mutations have revealed about the significant role that mutationally-activated *MET* plays in tumor initiation, progression, and therapeutic resistance.

MET kinase domain mutations in hereditary papillary renal carcinoma (HPRC)

The first activating mutations identified within the *MET* gene were discovered by a genome-wide analysis of families with HPRC (1). These seminal studies were the first genetic evidence demonstrating oncogenic activity of MET in humans. The germline missense mutations identified in HPRC patients (M1149T, V1206L, V1238I, D1246N, and Y1248C) flank the critical tyrosine residues Y1234 and Y1235 within the kinase domain (*Figure 1*). In addition, Schmidt et al. discovered somatic missense *MET* mutations (D1246H, Y1228C, and M1268T) within the same region in sporadic renal carcinomas (1,2). Cytogenetic studies revealed that the papillary renal carcinomas harboring *MET* mutations also contained trisomy of chromosome 7 (*MET* is located at 7q31). In each tumor, the Chromosome 7 triplication consisted of the non-random duplication of the chromosome harboring the mutated *MET* allele (3). The requirement for a second copy of the mutant *MET* allele in papillary renal carcinomas suggested that there is a necessary dose of activated MET required for tumor initiation in the kidney. Importantly, these findings revealed that mutated *MET* is a driver gene in hereditary and sporadic papillary renal carcinomas (SPRC).

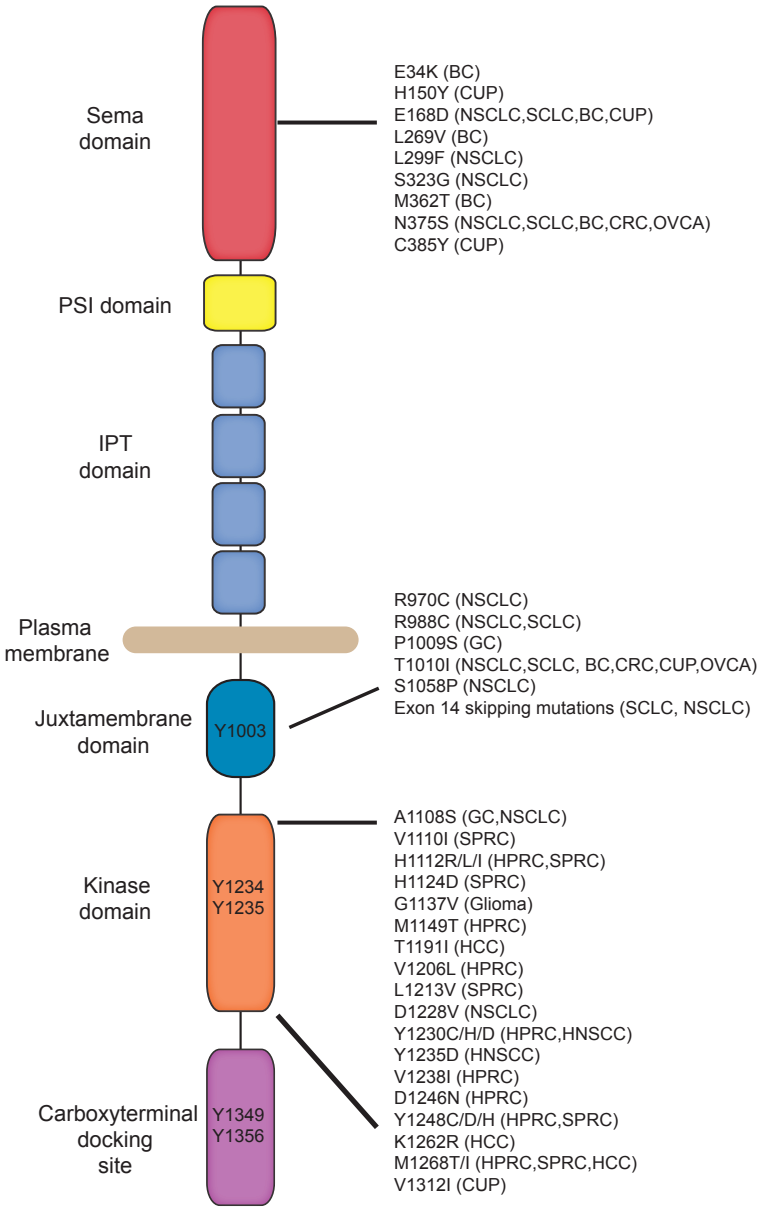

Figure 1 Mutations found within the functional MET domains. MET is expressed at the plasma membrane: the extracellular portion consists of the Sema domain, a PSI domain, and four immunoglobulin-plexin-transcription (IPT) repeats; the intracellular region contains the juxtamembrane domain, the tyrosine kinase domain and the carboxyterminal docking site. The cancer type in which mutations have been identified is noted in parentheses: breast cancer (BC), cancer of unknown primary origin (CUP), colorectal cancer (CRC), gastric cancer (GC), hepatocellular carcinoma (HCC), hereditary papillary renal carcinoma (HPRC), non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), and sporadic papillary renal carcinoma (SPRC). Note that the amino acid positions are based on the position number reported in the original publications. The numbering of several sites has been amended in more recent sequence reports (i.e., T1010I is often reported as T992I).

Understanding how mutations within the *MET* kinase domain affect activation and downstream signaling is vital for our understanding of dysregulated RTK signaling and for the development of effective kinase inhibitors. Several *in vitro*, xenograft, and transgene experiments verified the inherent oncogenic activity of the *MET* kinase domain mutations found in HPRC. These studies revealed that the kinase domain *Met* mutations induce constitutive receptor activation (2,4,5) and mutationally activated Met can be ligand-dependent or ligand-independent (5-8). Knock-in *in vivo* models of the kinase domain mutations were developed to characterize how mutationally activated MET effects tumorigenesis from initiation to malignant progression. Germline knock-in mouse models were created carrying unique *Met* kinase mutations including WT, D1226N, Y1228C, M1248T, and M1248T/L1193V (9,10). Interestingly the different mutant *Met* knock-in lines developed unique tumor profiles including carcinomas, sarcomas, and lymphomas. For example, Met^{M1248T} mice developed a mix of carcinomas and lymphomas while mice harboring D1226N, Y1228C, and M1248T/L1193V mutations developed a high frequency of sarcomas and some lymphomas. These mouse models also replicated the genomic events observed in human HPRC where nonrandom duplication of the mutant *Met* allele was observed in the majority of the tumors. Even though the knock-in mutation models never developed renal carcinomas, when placed on an FVB/N background, each *Met* mutant (except for D1226N) developed aggressive mammary carcinomas (11). Again, unique mammary carcinoma phenotypes were observed between the M1248T, Y1228C, and M1248T/L1193V lines (12). Since the only differences between these animals were the *Met* mutations and the murine background strain, this study indicated that either the mutated kinase structure itself or the level of kinase they impose (or both) influence the tissue-specificity for tumor formation. Overall, these studies demonstrated that the activating mutations affect more than just *MET* kinase activity and have the potential to drive tumorigenesis in numerous tissue types.

After the discovery of *MET* mutations in HRPC, studies in other solid tumors identified *MET* kinase domain mutations and some mutations outside the kinase domain in childhood hepatocellular carcinomas, breast cancer, colorectal cancer (CRC), head and neck squamous cell cancers (HNSCC), gastric carcinomas (GC), and cancers of unknown primary origin (CUP) (*Figure 1*) (13-21). For several years after the initial discovery, the small number of *MET* kinase activating mutations identified in other carcinomas suggested that mutations within the *MET* kinase domain were rare events in cancer. However, recent genomic screens have revealed that activating MET mutations are more frequent than initially thought (22) (COSMIC database at www.cancer.sanger.ac.uk/cosmic). The diversity of cancers in which *MET* mutations have been identified suggests that mutationally activated MET plays a significant role in the tumorigenic process in a wide range of cell types.

MET juxtamembrane and Sema domain mutations

Since the original screens for kinase domain mutations in other solid cancers identified few variants, searches expanded to regions outside of the kinase domain. In addition to the discovery of novel driver *MET* mutations, these findings have been critical for our understanding of receptor tyrosine kinase recycling and downregulation. The first juxtamembrane domain (JM) mutations were discovered in a gastric cancer (P1009S) and a breast cancer biopsy (T1010I) (Figure 1) (16). Though the importance and frequency of these mutations was later established in several lung cancer studies (23,24). In a sequencing analysis of small cell lung cancers (SCLC) and non-small cell lung cancers (NSCLC), Ma et al. identified missense mutations in the JM domain (R988C, T1010I, S1058P) and the Sema domain (E168D, L299F, S323G, and N375S) (23,25). A separate study in NSCLC and CRCs identified several mutations in the Sema (N375S) and the JM (R970C and T992I) domains (24). These JM domain mutations were shown to attenuate MET receptor ubiquitination and degradation and prolong MET signaling. Sema domain mutations have not been carefully evaluated but likely affects the structure of the ligand-binding domain (26). In addition, novel intronic mutations flanking exon 14 were discovered that result in an alternatively spliced *MET* transcript which encodes for a deletion of the JM domain (*METex14del*) (24,25). Like the JM domain mutations, *METex14del* receptor downregulation is abrogated by loss of the Cbl site on the JM domain and results in elevated, membrane expression of MET. Importantly, these studies uncovered a novel and distinct mechanism of oncogenic RTK activation through altered RTK downregulation (27,28). The incidence of *MET* mutations in lung cancers is 3% in squamous cell lung cancers, 5.6% in NSCLC, and 8% in lung adenocarcinomas (29-31). The implications of *MET* JM domain mutations and receptor regulation on

the clinical outcome of lung cancer patients have become evident in recent years (32-37). Moreover, the importance of these Sema and JM domain mutations is not limited to lung cancer. *MET* mutations have been detected in 9% of advanced breast cancer (20) and 7.4% advanced ovarian cancer patients (38). These studies underscore the role of mutationally activated MET in a wide range of cancers and the diverse mechanisms by which RTKs can achieve oncogenic activity.

Reemergence of MET kinase domain mutations during resistance

Tyrosine kinase inhibitors (TKIs) have had significant success in breast cancer (trastuzumab), melanoma (vemurafenib), and lung cancer (erlotinib), but in spite of these promising results, the clinical response to TKIs is often not durable. The efficacy of targeting kinases has been clearly demonstrated in lung cancer; however these studies have also highlighted the numerous mechanisms by which acquired resistance occurs. The presence of *EGFR* mutations, *ALK* fusions, and *MET* amplification in NSCLC has allowed for the clinical development of several therapeutic approaches using TKIs. For example, *EGFR*-mutant NSCLCs are sensitive to EGFR TKIs (gefitinib and erlotinib); however, resistance typically develops after 9–14 months (39,40). The most common mechanism of TKI resistance is a second-site mutation (T790M) in the *EGFR* kinase domain, however in approximately 20% of cases, EGFR inhibition leads to the expansion of subclones harboring amplified *MET*. Until recently, *MET* kinase domain mutations have been limited to papillary renal carcinomas, yet the use of TKIs in lung cancer revealed that MET kinase domain mutations may be a mechanism of therapeutic resistance in refractory lung cancer. In a study of a lung adenocarcinoma patient who had progressed on erlotinib treatment, both mutated *EGFR* and amplified *MET* were identified in the tumor. Treatment with combined MET and EGFR inhibitors (savolitinib and osimertinib) resulted in a dramatic clinical response (41). When resistance developed to combined MET and EGFR inhibition, a *MET* D1228V kinase domain mutation was detected. In a separate study, a NSCLC patient with a *METex14* deletion was treated with the MET inhibitor crizotinib and upon progression a D1228N mutation was detected (42). Likewise in NSCLC patient with a *MET*ex14 deletion, a Y1230C mutation was also detected at a very low frequency (mutant allele frequency =0.3%); however after 13 months of crizotinib treatment the tumor progressed and the Y1230C allele was detected in 3.5% of circulating tumor DNA (43). These results indicate that *MET* kinase domain mutations may be a mechanism to circumvent MET TKI inhibition. Several earlier studies have demonstrated the ability of *MET* kinase domain mutations to diminish the efficacy of MET inhibitors (44-46). It is likely that additional and novel mutations will be identified with the emergence of genomic profiling of recurrent disease through biopsies, circulating tumor cells, and circulating DNA. Therefore it is essential that we gain a clear understanding of how MET activating mutations alter the three-dimensional structure of the receptor in order to predict and develop effective MET inhibitors.

Summary

In summary, the search for *MET* mutations has uncovered the variety of cancers that deregulated MET affects from the early stages of tumor initiation to therapeutic resistance and recurrence. Moreover, the variety of mutations identified within the MET receptor has illuminated unique mechanisms of tumor initiation including nonrandom duplication of mutant alleles, intronic splice site alterations, and altered receptor downregulation. Advances in genomic screening and structural analyses are likely to shed additional light on the prevalence and functional activity of mutationally-activated *MET*. With this knowledge, MET-targeted therapies may benefit patients in a wide range of cancers.

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Footnote

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