Chapter 8
The MET Receptor Family

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8.1 Introduction to the Met Receptor Tyrosine Kinase Family

8.1.1 MET

MET was isolated in 1984 as a transforming gene from a human osteosarcoma cell line which became more transformed after exposure to the carcinogen *N*-methyl-*N*′-nitroso-guanidine (MNNG-HOS). While the *MET* gene was named for *met*hyl [1], it is more appropriate for its function in tumor *met*astasis, as revealed in the study [2]. The first isolated *MET* gene was a chimeric gene (TPR-MET) containing sequences encoding the kinase domain and c-terminus of MET fused to TPR (translocated promoter region), which encodes a dimerization leucine zipper motif [3]. The two sequences are brought together through chromosomal rearrangement between chromosome 1 (TPR, 1q25) and chromosome 7 (MET, in 7q31). Subsequent studies indicated that the *MET* gene encoded a receptor tyrosine kinase (RTK), but the receptor's ligand was unknown at the time [4]. Molecular biological and biochemical experiments identified hepatocyte growth factor (HGF) as the MET ligand [5]. The ligand HGF was identified as a mitogen for hepatocytes [6–8], and independently identified as a fibroblast-derived cytokine that dissociated epithelial cells (scatter factor, SF) [9]. The two proteins were later found to be the same and were then referred to as HGF/SF [10].

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The binding of HGF/SF to MET elicits a diverse series of cellular responses including proliferation, scattering/motility, invasion into extracellular matrix, and branching morphogenesis. MET is primarily expressed in epithelial cells, while its ligand HGF/SF is produced by surrounding mesenchymal cells. An HGF/SF–MET-mediated interaction between epithelia and mesenchyme is required for cell migration and organ formation during embryonic development. MET signaling also participates in angiogenesis, wound healing, and organ regeneration in adults. However, aberrant activation of MET signaling has been found in a large number of different cancer types (www.vai.org/met). Numerous experimental studies and clinical investigations have demonstrated that aberrant MET signaling contributes to tumor development and malignant progression.

8.1.2 RON

In 1993, another member of the MET family, RON, was cloned by screening a cDNA library prepared from human foreskin keratinocytes [11]. RON encodes a receptor tyrosine kinase that is structurally similar to MET (Fig. 8.1); the proteins share a 63 overall sequence identity in their intracellular regions [11]. The ligand for RON is a serum-derived growth factor, MSP (macrophage stimulating protein) [12–15], which belongs to the HGF/SF family [16]. The RON receptor and its ligand are involved in embryonic development and are crucial in regulating certain physiological processes [17]. Aberrant activation of RON through overexpression or alternative splicing has been reported in various tumor types. Moreover, transgenic expression of RON in lung epithelial cells resulted in tumors with the pathological features of human bronchioloalveolar carcinoma. Thus, RON plays an important role in human cancers and may be a target for therapeutic intervention. Since RON and MET are structurally and functionally similar [18], we summarize the two receptors in table “Receptor at glance: comparison between Met and Ron.” This chapter will focus on MET.

8.2 The MET Receptor and Its Ligand HGF/SF

8.2.1 Genomic Organization, Transcription, and Synthesis of the MET Receptor

The *MET* gene is at 7q31 and consists of 21 exons. The 5′-regulatory region of the MET promoter lacks TATA or CAAT elements, but it has an extremely high G-C content and multiple Sp1-binding sites [19]. Besides Sp1, several other transcription factors including HIF-1 [20], Ets1 [21], Pax3 [22], and AP1 [23] were

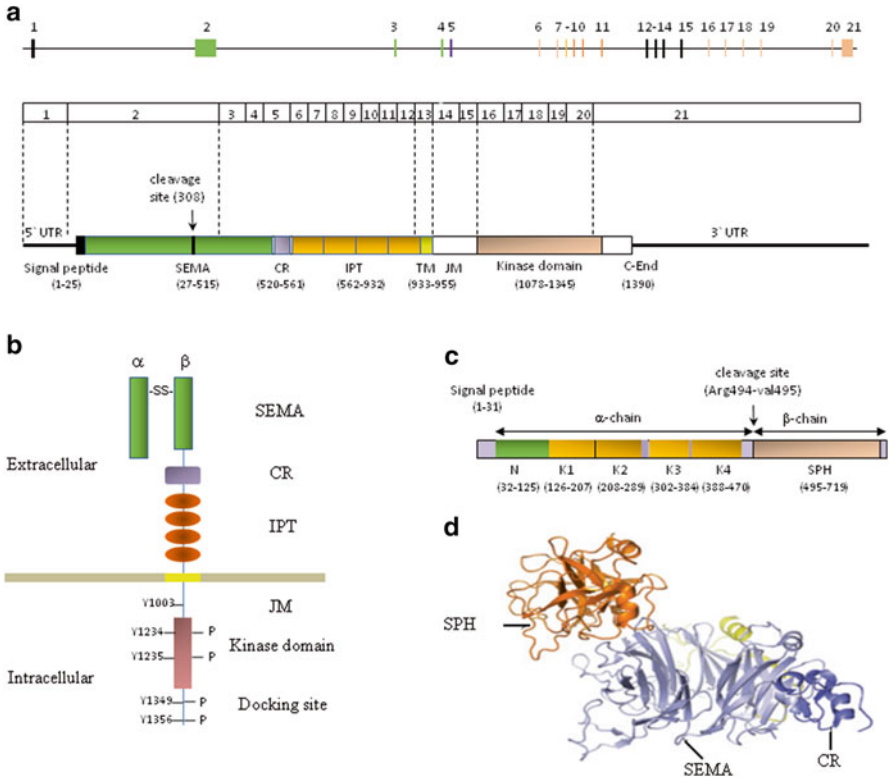

Fig. 8.1 Genomic structure and transcription of MET. (**a**) schematic representation of the *MET* gene locus. Exons are indicated by solid boxes and numbered above, while introns are indicated by the horizontal line. Numbered boxes indicate the exons of *MET*. MET protein is synthesized as a single-chain precursor and cleaved by furin during transit through the endoplasmic reticulum, thus yielding a smaller amino-terminal α-chain and a larger β-chain. (**b**) The MET ectodomain consists of a large N-terminal SEMA domain, which adopts a seven-bladed β-propeller fold and a stalk structure consisting of four immunoglobulin-like (Ig) domains. The SEMA domain and the stalk structure are separated by a small cystine-rich (CR) domain. The transmembrane (TM), the long juxtamembrane (JM) sequence, the kinase (K) domain, and a carboxy-terminal Docking site are also shown. (**c**) Hepatocyte growth factor/scatter factor (HGF/SF) is composed of six domains: an N-terminal (N) domain, four copies of the kringle domain (K1–4), and a C-terminal serine proteinase homology (SPH) domain that is structurally related to the catalytic domain of serine proteinases but that is enzymatically inactive. Mature, biologically active HGF/SF is a two-chain (α–β) protein that is produced by site-specific proteolysis in the extracellular space from single-chain pro-HGF/SF by the serine proteinases matriptase, hepsin, and HGF activator. HGF/SF contains two MET-binding sites: one in the NK1 fragment and one in the SPH domain. (**d**) The crystal structure of an SPH–MET complex is shown: the SPH domain of HGF/SF binds to an area of the SEMA domain within the MET α-chain (protein databank (PDB)

reported to be positive regulators of *MET* promoter. p53 was reported to be a transcription activator of MET, and a p53-responsive element was identified in *MET* promoter [24]. However, a later study showed that p53 could suppress MET expression at transcriptional and posttranslational levels in ovarian carcinoma cell lines, through inhibiting AP-1 and inducing miR-34, respectively [25]. The discrepancy may result from contextual difference of model systems used in the two studies.

Exon 1 of *MET* is noncoding and contains most of the 5′ UTR. Exon 2 is the largest internal coding exon (1,214 bp) in the *MET* gene and contains 14 bp of 5′ untranslated sequence followed by the initiating codon. Thus, the 4,170-bp open reading frame for the 1,390-amino-acid MET polypeptide precursor is distributed over 20 exons. After synthesis, the MET precursor undergoes proteolytic cleavage between Arg^{307} and Ser^{308}, forming an extracellular α chain and membrane-spanning β chain linked by disulfide bonds [26, 27]. Furin, a subtilisin-like mammalian endoprotease, has been identified as the processing endoprotease [28]. The 45 kDa α-chain is encoded by part of exon 2, whereas the 145 kDa β-chain is encoded by the rest of exon 2 together with exons 3–21 (Fig. 8.1).

The extracellular portion of the β subunit contains a semaphorin homology domain (SEMA), a cysteine-rich (CR) domain (also called the MET-related sequence, MRS), and four immunoglobulin-like (IPT, for IgG-like, plexins, transcription factors) domains. The intracellular portion of the β subunit contains a juxtamembrane domain (JM), a kinase domain, and a c-terminal docking site domain [29]. The HGF/SF-binding site is formed by the SEMA domain of the β-chain plus the α-chain [30]. The IPT domains 3 and 4 may be required for high-affinity binding between HGF/SF and MET [31]. The JM domain plays a key role in the binding of the CBL protein and in MET degradation. Phosphorylation of the JM domain at Y1003 is required for recruitment of CBL upon MET activation [32].

8.2.2 Genomic Organization, Transcription, and Synthesis of HGF/SF

The human HGF-encoding gene on chromosome 7q21.1 is composed of 18 exons and 17 introns. In the HGF/SF promoter region, an Sp1-binding site (at position –318 to –303 bp from the transcription start site) with a CTCCC motif has been identified [33]. Both Sp1 and Sp3 bind to this region and synergistically enhance HGF/SF gene expression. Other regulatory elements, CCAAT/enhancer-binding protein beta (C/EBP-β) and delta (C/EBP-δ), are located between –6 and +7 bp from the transcription start site. The core binding sequence for the inducible cis-acting factors was TTTGCAA (–4 to +3 bp). Partial hepatectomy increases C/EBP

binding activity to this region, providing a mechanistic explanation for the transcriptional induction of HGF/SF by extracellular signals (i.e., cytokines) that induce tissue regeneration [34]. A DNA element consisting of a mononucleotide repeat of 30 deoxyadenosines (deoxyadenosine tract element, DATE) is identified at 750 bp upstream from the transcription start site in the human *HGF* promoter. DATE acts as a transcriptional repressor, whose truncation leading to constitutive activation of the *HGF* promoter. DATE is a target of deletion in human breast cancer cells and tissues [35].

Expression of the *HGF* gene has been found to be upregulated by various cytokines and growth factors, including IL-1, TNF-α, EGF, FGF, and PDGF [36, 37], as well as by prostaglandins [38] and heparin [39]. In contrast, HGF expression is downregulated by dexamethasone and transforming growth factor β1 [40, 41]. *HGF* expression is restricted to non-epithelial cells, such as the fibroblasts of various tissues, Ito cells of the liver, macrophages, peripheral blood leukocytes, endothelial cells, and megakaryocytic cells [42].

HGF/SF is produced predominantly in mesenchymal cells as a precursor of 728 amino acid residues, which is mostly found in extracellular matrix [7]. While pro-HGF binds to MET with a high affinity, it is unable to activate MET [43]. Pro-HGF is proteolytically processed at Arg^{494}-Val^{495} to generate mature HGF/SF, a disulfide-linked heterodimer composed of a 69 kDa α subunit and a 34 kDa β subunit [44]. The α subunit contains a hairpin loop followed by four kringle (K1–K4) domains and is highly homologous to members of the plasminogen serine protease family. The β subunit resembles a serine protease homology domain (SPH), but lacks protease activity, partly due to mutations in residues forming the serine protease catalytic triad. The first kringle domain in the α-chain contains the high-affinity binding domain for MET [43, 45].

8.2.3 Activation of HGF/SF by Serine Proteinases

Three serine proteinases have been implicated in the activation of pro-HGF/SF: HGF activator (HGFA), matriptase (ST14), and hepsin. Matriptase and hepsin are type II transmembrane enzymes that efficiently activate pro-HGF at the cell surface [46]. In contrast, HGFA was originally isolated from bovine serum as a soluble proteinase capable of HGF/SF activation [47]. HGFA is present in human plasma as an inactive zymogen, which is processed by thrombin. The activated HGFA has a molecular mass of 34 kDa and consists of two chains held together by a disulfide bond [48]. The nucleotide sequence of the cDNA reveals that HGFA precursor protein contains 655 amino acid residues and consists of multiple putative domains homologous to those observed in blood coagulation factor XII [49]. Coagulation factor XIIa also has the ability to activate single-chain HGF, although

the specific activity is slightly lower than that of HGFA. The involvement of thrombin, a component of blood coagulation cascade, in HGFA activation suggests that HGFA is a key enzyme for HGF/SF activation during tissue regeneration [50, 51]. This idea is further supported by a study of HGFA-deficient mice, which exhibit decreased activation of HGF/SF and impaired regeneration of injured intestinal mucosa [52].

The activation of HGF/SF is finely tuned by two HGFA inhibitors: HGF activator inhibitor type 1 (HAI1; also known as SPINT1) and type 2 (HAI2; also known as SPINT2) [53, 54]. Both inhibitors are synthesized as integral membrane proteins containing two Kunitz domains and a transmembrane domain, and they are subsequently released by shedding from cell surface [55]. The inhibitors also inactivate matriptase, which is required for maintaining epithelial integrity [56], as well as for placental and neural development [57]. HGF/SF is known to associate with components of the extracellular matrix, including heparan sulfate proteoglycan, thrombospondin, fibronectin, and vitronectin [58, 59]. Matrix metalloprotease-mediated extracellular matrix degradation, which is triggered by the uPA/uPAR-plasmin system, facilitates the release and activation of sequestered pro-HGF from the extracellular matrix [60].

8.3 HGF/SF Activation of MET

8.3.1 Activation and Signaling

Binding of HGF/SF to MET receptor triggers dimerization and phosphorylation of the receptor. Phosphorylation at two tyrosine residues, Y1234 and Y1235, in the catalytic domain is crucial for activating MET as a tyrosine kinase [61], while phosphorylation at Y1349 and Y1356 in the C-terminal portion of the molecule is essential for its functioning as a docking site. Upon phosphorylation, the docking site recruits the Src homologous 2 (SH2)-domain-containing proteins, which in turn trigger specific signaling (Figs. 8.1 and 8.2). The adaptor proteins and signal transducers that physically bind to the phosphorylated MET receptor include Grb2 [29], Gab1 [62], SHC [63], Src [29], PI3K [64], and STAT3 [65].

Grb2 was isolated as a growth factor receptor-bound protein that contains a Src homology 2 (SH2) domain between two SH3 domains [66, 67]. The SH2 domain associates with the growth factor receptor, while the SH3 domains interact with the carboxyl-terminal domain of SOS (Son of Sevenless) to mediate RAS signaling [68–70]. The interaction between MET and Grb2 may be enhanced by SHC, which is recruited to the MET docking site and phosphorylated by activated MET. The phosphorylation of SHC produces a binding site ($pY^{317}VNV$) for Grb2 [63, 67]. Interestingly, the interaction between MET and SHC requires α6β4 integrin, which also physically interacts with MET [71]. Activated RAS triggers the activation of the MAPK pathway through RAS-RAF-MEK1, MEK2. This pathway is required

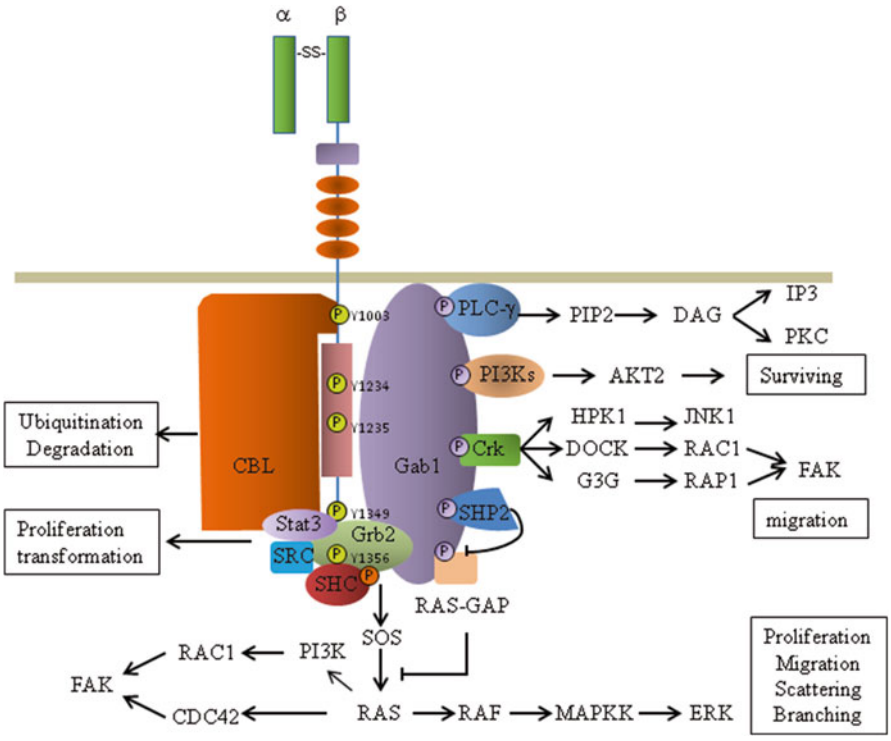

Fig. 8.2 Signaling pathways from HGF/SF activation of c-MET receptor. HGF/SF binding triggers MET dimerization and autophosphorylation activity. Phosphorylation at Tyr1234 and Tyr1235 of the kinase domain activates tyrosine kinase activity of MET. The phosphorylation of Tyr1349 and Tyr1356 at the docking site results in recruitment of various cytoplasmic effector molecules GRB2, GAB1, PLC, and SRC. Tyrosine-phosphorylated GAB1 that is bound to MET can attract further docking proteins, including SHP2, PI3K, and others. Phosphorylation and activation of these adaptors activate various downstream signaling cascades. Activation of MAPK results from sequential activation of several protein kinases including SOS, RAS, RAF, and MAPKK. PI3K is a lipid kinase catalyzing the formation of PIP3, which creates a docking site for Akt to the inner side of the plasma membrane. Activation of Akt leads to phosphorylation and activation of several substrates involved in cell proliferation and surviving. Phosphorylation of MET at Tyr1003 of JM domain results in the binding of CBL, an E3 ligase that triggers MET ubiquitination and degradation

for cell proliferation induced by growth factors, but it is also involved in other effects of MET signaling. For example, the activation of the MAPK pathway by MET induces the expression of urokinase, which plays an important role in cell invasion [72–74].

The role of Grb2 in MET-induced branching morphogenesis has been suggested from the use of mutant MET molecules that selectively disrupt the association: mutation at the consensus Grb2-binding site on MET, N1358H, disrupts the interaction between Grb2 and MET. Cells expressing this mutant receptor can scatter but

are unable to form branching tubules [75–78]. The role of Grb2 for the migration of muscle precursor cells in late myogenesis is also suggested by studies using this *MET* mutant as a mouse germline knock-in. These animals showed a striking reduction in limb muscle formation, while the development of placenta and liver was unaffected relative to animals nullizygous for *MET* [78].

Gab1 (Grb2-associated binder-1) was originally discovered as a Grb2 interacting protein that shares homology and structural features with IRS-1 (insulin-receptor substrate-1) [79]. Grb2 binds to Gab1 via its SH3 domain and to MET via its SH2 domain, thus coupling Gab1 to the MET receptor. Gab1 also directly binds to MET through its phosphotyrosine recognition domain (or MET-binding domain, MBD) [62]. Gab1 mutants deficient in Grb2 binding associate with MET but with a reduced strength, indicating that both direct and indirect binding are essential [80]. The N-terminal pleckstrin homology (PH) domain that binds phosphatidylinositol 3,4,5-triphosphate is critical for subcellular localization of Gab1. A Gab1 mutant lacking the PH domain is localized predominantly in the cytoplasm and loses the ability to induce branching morphogenesis [81]. Upon stimulation with HGF/SF, Gab1is recruited to the MET receptor and is phosphorylated at several tyrosine residues, which in turn recruit downstream adaptors and signaling molecules such as tyrosine phosphatase SHP2, PI3K, PLC-γ, and Crk/CRKL [80]. The specific tyrosine phosphorylation patterns on Gab1 specify the binding of different downstream molecules. For example, the phosphorylation of Y447, Y472, and Y589 is required for binding to the regulatory subunit p85 of PI3K [81, 82]; of Y627 for binding to SHP-2 [82, 83]; and of Y307, Y373, and Y407 for binding to PLC-γ [84].

The functions of SHP-2 and PLC-γ in MET signaling have been characterized by using Gab1 that is mutated at specific tyrosine residues required for its binding with distinct targets. The Gab1 C-terminal mutant Y637F fails to recruit SHP-2 and is unable to elicit sustained activation of ERK and epithelial morphogenesis in response to HGF/SF [80, 85]. As a tyrosine phosphatase, SHP2 may enhance RAS/ERK signaling by dephosphorylating the RAS-GAP-binding site on Gab1 and disengage RAS-GAP to sustain RAS activation [86]. A recent study showed that SHP2 deficiency compromises the mitotic checkpoint and results in chromosome instability and cancer predisposition. SHP2 is required for the optimal activation of the mitotic kinases PLK1 and Aurora B and thereby the proper kinetochore localization and phosphorylation of BubR1 [87]. Overexpression of the Gab1 mutant molecule Y307/373/407F, which is unable to bind PLC-γ, completely abolished HGF/SF-mediated tubulogenesis without altering scattering and only partially reduced cell growth [84].

Gab1 also contains multiple Tyr-*X*-*X*-Pro (Y*XX*P) motifs that bind to the adapter proteins c-Crk and Crk-like (CRKL) upon HGF/SF treatment [88]. c-Crk and CRKL are SH2- and SH3-domain-containing proteins, with the SH2 domain binding to Gab1 and the SH3 domain recruiting downstream adaptors including C3G, DOCK180, and HPK-1[89–91]. C3G is a guanine-nucleotide exchange factor that activates Rap1 [92], which in turn controls adherent junction positioning and cell adhesion [93]. DOCK180 is an activator of Rac1, which mediates MET-induced cell spreading and migration [94]. HPK-1 (hematopoietic progenitor

kinase1) is a well-established activator of JNK that is essential for MET-induced transformation [95–97].

PI3K is another Gab1-binding molecule that has been linked to HGF/SF-induced proliferation, scattering, and branching morphogenesis [98–100]. The PI3K/AKT pathway is a key to mediating cell survival in response to DNA damaging agents or serum starvation [101–103]. Survival signals emanating from HGF/SF–MET are enhanced by caspase-cleavage products of GAB1, a p35-GAB1 fragment that favors cell survival by maintaining HGF/SF-induced MET activation of AKT [104]. MET also mediates cell survival in PI3K/AKT-independent manner. For example, MET can prevent Fas-induced apoptosis by directly binding to Fas and blocking its self-aggregation and its ligand binding [105].

SHIP-1 (SH2-domain-containing inositol 5-phosphatase 1) was originally identified as a negative growth regulator in cytokine-stimulated hematopoietic cells [106]. In yeast two-hybrid screening, SHIP-1 was discovered to be a MET-binding protein [107]. MDCK cells that overexpress SHIP-1 branch early relative to wild-type cells in response to HGF/SF, while a mutant SHIP-1 molecule lacking catalytic activity impairs HGF/SF-mediated branching morphogenesis [107].

Upon HGF/SF activation of MET, Src binds to MDS domain of MET, which results in Src phosphorylation and activation. Activation of Src is required for HGF/SF-induced cell transformation [29]. HGF/SF also stimulates the recruitment of STAT-3 to MET receptor, which is followed by its tyrosine phosphorylation and nuclear translocation. STAT-3 is a transcription factor that activates the expression of genes required for HGF/SF-induced branching morphogenesis [65], or anchorage-independent growth and tumorigenic activity [108]. Also, Src and STAT-3 may cooperate to upregulate HGF expression [109].

8.3.2 Modulation of MET Activation by Other Signal Molecules

While HGF/SF is the only known ligand for MET, a number of signal molecules have been implicated in effective activation of MET. These proteins that augment MET activation include CD44, integrin, class B plexins, and other RTKs.

CD44 is a receptor for hyaluronic acid that is involved in cell–cell interactions, cell adhesion, and migration. CD44 exists in multiple isoforms that are generated through alternative splicing. CD44 isoforms containing the alternatively spliced exon v3 (CD44v3) carry heparan sulfate side chains that are able to bind HGF/SF. CD44v3 may enhance MET signaling by concentrating and presenting HGF/SF to MET [110]. Co-expression of CD44v3 and MET correlates with a poor prognosis of colon cancer, suggesting CD44v3 may promote HGF/SF-induced tumor progression [111]. Another isoform, CD44v6, forms a complex with HGF/SF–MET that enhances HGF-dependent MET phosphorylation [112] and activation of MAPK pathway in several tumor cell lines [112, 113].

The collaboration of CD44 and MET is required for development of the central and peripheral nervous systems; mice with MET (and HGF/SF and Gab1) heterozygous mutations on a $CD44^{-/-}$ background die at birth with defects in nervous system development. However, CD44-null animals or animals heterozygous for MET do not exhibit these defects, probably because ICAM-1 (intercellular adhesion molecule-1) can compensate for CD44 as a co-receptor for MET in CD44-null mice. In CD44 wild-type mice, MET activation and cell proliferation following partial hepatectomy were inhibited by CD44v6-specific antibodies, but ICAM-1-specific antibodies only interfered with liver cell proliferation and MET activation in CD44 knockout mice [114, 115]. These studies indicated that cross talk between CD44 and HGF/SF–MET signaling plays an important role in adult physiology and embryonic development.

Integrins are a group of membrane proteins that mediate the attachment of cells to the extracellular matrix. Certain integrins, such as α6β4, selectively associate with MET and potentiate HGF-triggered activation of the RAS and PI3K-dependent pathways [116]. Integrin-mediated cell–matrix adhesion may also activate MET in the absence of HGF/SF [117, 118]. The cross talk between integrin and MET may synergistically promote tumor invasion.

Plexins are single-pass transmembrane receptors for semaphorins, which modulate cytoskeletal remodeling and integrin-dependent adhesion [119]. Class B plexins and MET share homology in their extracellular domains: they both contain a Sema domain that forms a β-propeller structure, a cysteine-rich motif, and immunoglobulin-like domains [119]. The propeller domain mediates MET association with class B plexins [120–122]. The binding of Sema4D to plexinB1 increased MET signaling and enhanced cell invasion, while MET expression was also required for effective activation of plexinB1 by Sema4D [121].

The cross talk between HGF/SF–MET and other signaling molecules is also required for embryonic development. The absence of MET during renal development causes reduced branching of the ureteric bud and a decreased number of nephrons. Mice missing both MET and EGFR exhibit more serious defects in renal development [123], suggesting that cross talk between MET and EGFR family members is likely to be important [123–125]. Functional cross talk between MET and EGFR has been reported in several systems [126, 127]. Co-expression of MET and Her-2 is often detected in breast and gastric cancer cells [128, 129]. EGF stimulation of bladder, hepatocyte, epidermoid carcinoma, and non-small cell lung cancer cell lines activated both EGFR and MET [126, 130, 131]. In contrast, EGFR inhibition by Gefitinib significantly blocks HGF/SF activation of MET and the HGF/SF-induced proliferation and migration of mammary carcinoma cell lines [132], suggesting that the EGF/EGFR ligand/receptor pair is required for full activation of MET signaling. On the other hand, HGF/SF promotes transactivation of EGFR during retinal pigment epithelial wound healing, leading to an enhanced activation of downstream signaling pathways [133]. Activation of MET through amplification in lung cancer cells activates the ERBB3–PI3K pathway and promotes resistance to EGFR kinase inhibitors [134]. Therefore, the cross talk between MET and EGFR is an important mechanism for cancer progression and resistance to therapy.

Cross talk between MET and WNT–β-catenin occurs at several levels. First, MET can contribute to the transcriptional activation of WNT ligands such as WNT7B [135]. Second, MET can also stabilize β-catenin by inhibiting its degradation through AKT phosphorylation of glycogen synthase kinase-3β (GSK3β). Third, HGF/SF activation of MET promotes nuclear translocation of β-catenin and the transcription of their target genes in liver and bladder cancer cells [136]. On the other hand, MET is a direct transcriptional target of WNT–β-catenin in colon cancer cell lines [137].

8.3.3 MET Internalization, Processing, and Attenuation

The strength and duration of MET activation is tightly regulated to induce appropriate cellular responses [138]. Levels of MET expression at the cell surface are finely tuned by multiple mechanisms, including clathrin-mediated endocytosis, extracellular shedding, and intracellular cleavage. Downstream signaling is also restricted through negative feedback loops.

While HGF binding activates MET signaling, it also triggers the downregulation of MET through receptor-mediated endocytosis [139]. The proteasome activity seems to be necessary for MET internalization, although the detailed mechanism of how the proteasome participates is unknown [140, 141]. In this process, the MET-ligand complex is recruited to clathrin-coated pits, followed by internalization and endosomal trafficking, and ending with degradation in lysosomes or recycling to the plasma membrane [141]. Like other RTK receptors, the internalized receptor that is delivered to endosomal compartments remains capable of signaling during vesicle trafficking [142, 143] and is even required for certain signaling events such as the activation of ERK [144–146]. MET-activated ERK signaling within endosomal compartments is regulated by PKC-ε, which ensures the consequent accumulation of ERK in focal complexes [146]. In contrast, PKC-α is only required for the microtubule-based movement of MET from an early endosomal compartment to a perinuclear compartment. MET being delivered to a perinuclear endosomal compartment seems to be required to sustain phosphorylated STAT3 in the nucleus [147]. Thus, the route of trafficking can determine the nature of the signal output.

The proto-oncogene CBL plays a key role in MET ubiquitination and degradation. CBL is an E3 ubiquitin ligase that serves as a negative regulator for a number of receptor tyrosine kinases [148–150]. In addition to a RING finger domain that recruits E2 enzyme, CBL contains a tyrosine kinase binding domain, which recognizes the phosphorylated Tyr1003 residue in the juxtamembrane domain of MET, and a proline-rich domain that binds to Grb2. The site of ubiquitin binding is at the C-terminal ubiquitin-associated domain (UBA) [32, 151, 152].

Upon MET activation, CBL is recruited to MET through Y1003 and is subsequently phosphorylated by MET to activate its E3 ligase activity. MET is then ubiquitinated [153] and recruited to the endophilin-CIN85-Cbl complex in clathrin-coated pits [154]. Formation of the endophilin-CIN85-Cbl complex triggers invagination

and scission of the membrane to form early endosomes. After endocytosis, the ubiquitinated MET receptors are retained in endosomes through their interaction with the ubiquitin-interacting domain contained in the *h*epatocyte growth factor-*r*egulated tyrosine kinase *s*ubstrate (HRS) [155, 156]. HRS couples ubiquitinated MET to the endosomal sorting complex for transport (ESCRT) to initiate formation of the multivesicular body, which is then targeted to the lysosomes for degradation [157]. The endosomal sorting process also requires the signal-transducing adaptor molecule (STAM) that forms a heterodimeric complex with HRS [158]. The unubiquitinated MET interacts with GGA3 via the CRK adaptor and ARF6. The formation of a GGA3-MET complex promotes access of MET into a recycling pathway. GGA3-dependent entry of MET into the recycling pathway promotes sustained ERK1/2 activation [159].

Cbl-dependent ubiquitination is crucial to targeting the MET receptor to components of the lysosomal sorting machinery, but it appears to be dispensable for MET internalization [160]. Thus, MET with mutation or deletion of the CBL-binding site is still internalized upon ligand activation, but it escapes degradation owing to a change in endosomal sorting [32, 161]. Such receptor variants lead to sustained signaling and convert MET into a transforming protein [162]. Beyond mutations in the JM domain, MET mutations in the kinase domain (D1246N and M1268T) produce increased endocytosis/recycling activity and decreased degradation of MET, which leads to the accumulation of MET in endosomes [163]. Endosomal MET activates the GTPase Rac1, which is required for cell migration, tumorigenic activity, and experimental metastasis [163].

Another mechanism that leads to downregulation of MET involves the proteolysis and shedding of the extracellular domain. Shedding is mediated by members of the disintegrin and metalloproteinase (ADAM) family which generate a soluble MET ectodomain and a membrane-anchored cytoplasmic tail. The cytoplasmic tail undergoes proteolysis by γ-secretase and is rapidly cleared by proteasome-mediated degradation [164]. Unlike Cbl-mediated endosomal degradation, proteolysis of MET does not require the ligand-mediated activation of MET. The extracellular shedding of MET not only decreases the number of receptor molecules on the cell surface but also generates a decoy moiety that interacts with both HGF and full-length MET to further inhibit MET signaling [165]. In an immortal trophoblast cell line, B6Tert-1, HGF/SF–MET signaling induces ADAM10 and ADAM17, which in turn lead to proteolysis and MET shedding. Thus, HGF/SF could self-control its regulation on trophoblast cell invasion by enhancing proteolysis of its receptor. Interruption of this feedback loop may impede placentation during mammalian placental development [166].

MET signaling can be inhibited by downstream molecules of its signaling pathway. Spry2 was first identified as an inhibitor of the FGF and EGFR signaling pathways during *Drosophila* organogenesis [167, 168]. Subsequent study indicated that Spry2 is transcriptionally upregulated in cells treated with HGF/SF, and its expression inhibits MET signaling and HGF/SF-induced cellular responses [169]. MET activation also leads to transcriptional induction of the Notch ligand Delta and the Notch effector HES-1. The activation of Notch signaling downregulates the MET

receptor and suppresses RAS-ERK signaling [170]. Loss of Spry2 leads to activation of RAS-ERK signaling and contributes to tumorigenesis, indicating that the counter-regulatory mechanism is required for appropriate function of MET signaling [171–173].

8.4 Cellular Responses to HGF/SF

Activation of MET signaling induces various cellular responses including cell growth [6, 174], scattering/migration [9, 175], invasion [176], tubulogenesis/branching morphogenesis [124], and lumen formation [177, 178].

8.4.1 HGF/SF–MET Signaling in Cell Proliferation

Growth factor-induced cell proliferation is defined by its capacity to induce DNA synthesis in quiescent cells [179]. Indeed, HGF/SF was first identified based on its capacity to stimulate DNA synthesis [8]. The most sensitive method to measure HGF/SF-induced DNA synthesis is [^{3}H]-thymidine incorporation (Fig. 8.3a). Serum starvation before HGF/SF treatment may be required to measure the effect of HGF/SF on DNA synthesis in cultured tumor cells, since serum is a strong stimulator. The time to reach the peak of DNA synthesis after HGF/SF treatment may be cell type dependent. In the case of SK-LMS-1 cells, DNA synthesis peaks at 12 h of HGF/SF treatment.

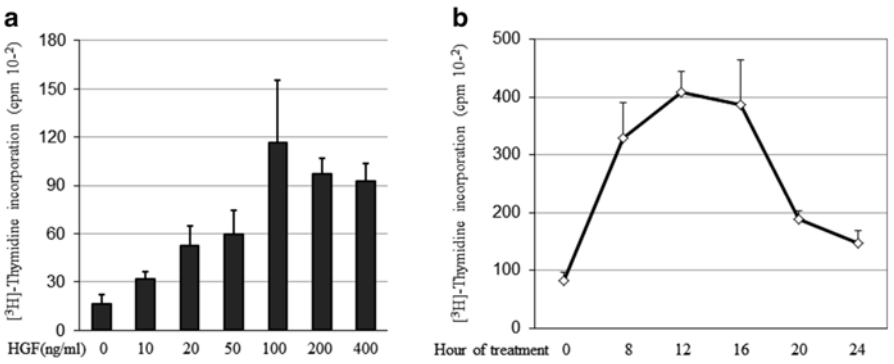

Fig. 8.3 HGF/SF stimulates proliferation on SK-LMS-1 cells as analyzed by [^{3}H]-thymidine incorporation assay. (**a**) Effects of HGF/SF on DNA synthesis. Cells were seeded in 96-well plate (2,000 cells/well) and cultured for 24 h. After serum starvation, the cells were treated with HGF/SF for 10 h. [^{3}H]-thymidine was added for 8 h before analysis. (**b**) DNA synthesis at various times after HGF/SF treatment. Cells were seeded in 96-well plate (5,000 cells/well) and cultured for 24 h. After serum starvation, the cells were treated with HGF/SF for various times. [^{3}H]-thymidine was added for 5 h before analysis

While the upregulation of cyclinD and downregulation of p27 through the RAS/MAPK and PI3K pathways are commonly involved in growth factor-induced cell growth [179], the activation of RAS/MAPK and PI3K is insufficient for HGF-induced growth, at least in some systems [180]. Activation of additional signaling, such p38 and NF-κB, may be also required [181, 182]. HGF/SF can induce proliferation through c-Myc in a proliferative subclone isolated from the DBTRG-05MG glioblastoma cell line [183]. The levels of phosphorylated ERK and AKT in the proliferative subclone were much lower than those of invasive subclones, which also exhibited low levels of c-Myc. This study suggests that high ERK and AKT activity is not required for c-Myc induction and proliferative response, although a basal level may be essential. Indeed, a high-intensity ERK signal mediates HGF/SF-induced proliferation inhibition in the human hepatocellular carcinoma cell line HepG2 [184, 185]. The role of Src in HGF/SF-induced proliferation was investigated using a Gab1 mutant having substitutions in the Src phosphorylation sites (Y242, Y259, Y317, and Y373). These Gab1 mutants failed to promote HGF-induced DNA synthesis but retained the ability to facilitate HGF-induced chemotaxis, indicating that Src is important for HGF-induced DNA synthesis [186].

8.4.2 HGF/SF–MET Signaling in Cell Scattering and EMT

The epithelial–mesenchymal transition (EMT) is a process characterized by loss of intercellular junctions and increased cell motility. In two-dimensional culture, EMT was reflected in cell spreading; a series of processes including disruption of cell–cell junctions; and subsequent cell scattering and migration [187]. HGF/SF was independently identified as scatter factor (SF), which causes a disruption of junctions, an increase in local motility, and a scattering of contiguous sheets of epithelial cells [9]. The role of HGF/SF in cell scattering is best manifested in MDCK cells (Fig. 8.4).

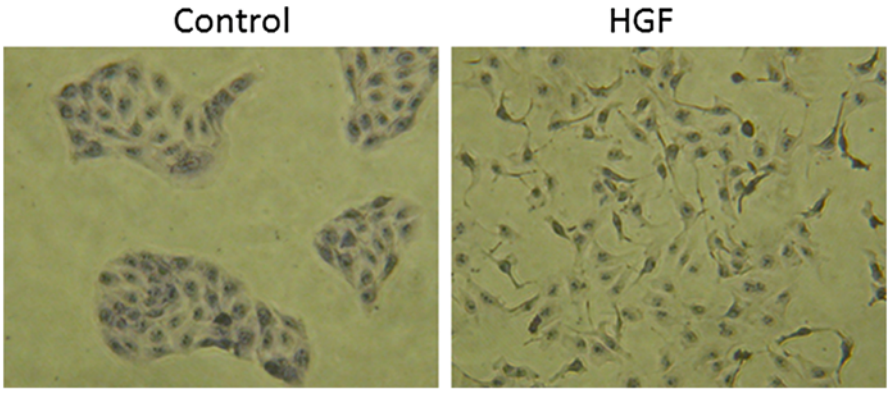

Fig. 8.4 HGF/SF-induced cell scattering in MDCK cells. Cells were treated with HGF/SF at 20 ng/ml for 24 h (HGF), or untreated (Control). Images were taken after cell staining with 0.005 % crystal violet

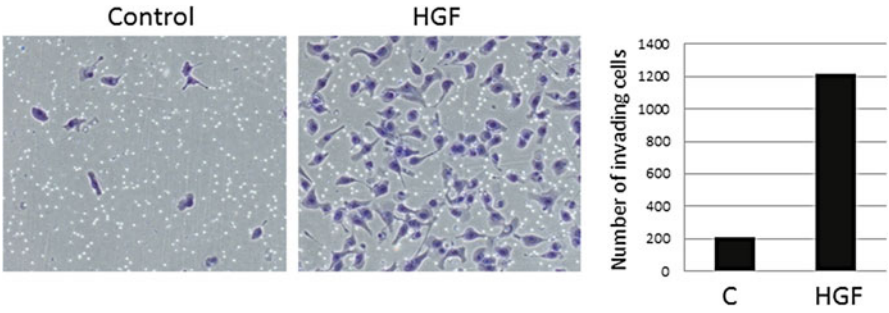

Fig. 8.5 HGF/SF-induced cell invasions through Matrigel. SK-LMS-1 cells (10,000 cells/chamber) were loaded into Boyden chamber and treated with HGF/SF at 100 ng/ml for 24 h (HGF), or were untreated (Control). Cells remaining inside the chamber were removed. Cells invading through Matrigel and attached to the bottom surface were stained. The number of invading cells in the whole insert was counted and presented in bar graph (unpublished data provided by Dr. Gao)

The RAS activation is sufficient for cell spreading and disruption of adherent junctions, while p42/p44 MAPK, PI3-kinase, and Rac are required for the downregulation of E-cadherin and the disruption of adherent junctions in MDCK cells [188]. The downregulation of E-cadherin may result from co-endo/exocytosis with MET [189]. HGF/SF-induced scattering may be a prerequisite for cell invasion; the process also needs the upregulation of uPA/uPAR [74] and members of the matrix metalloproteinase family [72, 73, 190–192] (Fig. 8.5). These studies suggest that HGF/SF-induced EMT may play a role in invasion and metastasis in human cancer. In fact, high level of circulating HGF/SF are associated with EMT in tumor tissue from small cell lung cancer and with poor outcome in patients [193].

EMT has been implicated in numerous developmental processes, including mesoderm formation and neural tube formation. HGF/SF–MET signaling is essential for the generation of myogenic precursor cells from the epithelial dermomyotome (i.e., EMT) as well as for the migration of myogenic precursor cells into the limbs, tongue, and other organs, where they differentiate to form a subset of the hypaxial muscles. The long-distance migration in the embryo is dependent on both MET and GAB1 [194, 195].

8.4.3 HGF/SF–MET Signaling in Tubulogenesis/Branching Morphogenesis

Tubulogenesis/branching morphogenesis refers to the organization of epithelial cells into branched tubular structures [196]. Branching morphogenesis is the structural basis for the formation of a variety of parenchymal organs, such as the kidney, liver, lung, and mammary gland during embryonic development.

Under physiological conditions, this is a highly complex process that involves the interaction of different cell types and is induced by various environmental cues. This process can be mimicked, in vitro, by culturing Madin-Darby canine kidney (MDCK) epithelial cells in three-dimensional (3D) collagen matrix in the presence of either fibroblasts or fibroblast-conditioned medium [197]. HGF/SF was subsequently identified as the sole growth factor responsible for branching morphogenesis [124]. None of the other known growth factors, including epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), transforming growth factor beta 1 (TGF-β1), insulin-like growth factor I (IGF-I), insulin-like growth factor II (IGF-II), platelet-derived growth factor (PDGF), or keratinocyte growth factor (KGF) displayed the same activity in MDCK cells [197]. It was subsequently shown that HGF/SF induces branching morphogenesis in collagen matrix in a wide variety of epithelial cells from colon, pancreas, mammary gland, prostate, lung, and other organs [198]. The tubular structures formed in vitro culture system resemble the epithelial organization of the organ of origin, indicating that HGF/SF can induce morphogenesis in diverse epithelial cells, and the exact morphogenic events are determined by the intrinsic programs of the epithelia [198].

HGF/SF-induced branching morphogenesis includes a series of steps: starting from spheroid cysts of MDCK cells cultured in collagen matrix, HGF/SF stimulation induces membrane protrusions of individual MDCK cells in the cyst that extend into the extracellular matrix. The protrusions then develop chains of cells that are connected to the cyst. Next, the chains form cords that are two to three cells thick and develop discontinuous lumens. Finally, the discontinuous lumens grow and fuse to become continuous with the lumen of the cyst [199, 200]. These processes required a series of cellular responses including invasion, proliferation, migration, survival, and differentiation. Therefore, a sequential and coordinated activation of signaling is required for each of the cellular responses [196]. For example, activation of the RAS–MAPK pathway is required for HGF/SF-induced early steps of tubulogenesis when cells form protrusions, proliferate, migrate, and organize themselves into long chains, but is dispensable for the later dedifferentiation steps where polarity is reestablished and a fluid-filled lumen is formed [201]. The strength of MAPK activation is also critical for branching morphogenesis [127]. HGF/SF stimulates complete breakdown of cell–cell junctions to generate single cells in MDCK cells expressing constitutively activated ErbB2/Neu receptor (NeuNT). Those single cells do not form cell chains and cords, which are necessary steps for branching morphogenesis. HGF-induced cell dispersal of NeuNT-expressing cells is lessened by pretreatment with a pharmacological inhibitor of the mitogen-activated protein kinase kinase (MEK) pathway, which restores cell–cell junctions and branching morphogenesis [127]. This study suggest that moderate MAPK activity and partial EMT are required for generating cell chains and cords in early stage of branching morphogenesis.

MDCK cells forms tubes when cultured in Type I collagen gels, but not in basement membrane Matrigel [202], indicating that the interaction between cell membrane and the components within extracellular matrix (ECM) plays a key role in this

process. By adding back individual components Matrigel to MDCK cells grown in Type I collagen gels in the presence of HGF, it has been shown that certain ECM proteins, such as Type IV collagen, heparan sulfate proteoglycan, and vitronectin, caused marked inhibition of HGF-induced morphogenesis. However, other components in Matrigel, such as laminin, entactin, and fibronectin, actually facilitated the formation of branching tubular structures and increased their complexity [202]. It is worth noting that the stimulating or inhibitory effect of an ECM component on branching morphogenesis may be cell type dependent, since many tumor cell lines exhibit branching morphogenesis in 3D Matrigel [203–205] (Fig. 8.6).

HGF/SF-induced branching morphogenesis can be modulated by various signal molecules or microenvironmental factors. For example, EphA2 acts as a positive regulator for HGF/SF-induced mammary epithelial branching morphogenesis, since the HGF/SF-dependent morphogenesis was significantly reduced in EphA2-deficient cells relative to wild-type cells. The branching defects can be rescued by inhibition of Rho-Associated, Coiled-Coil-Containing Protein Kinase (ROCK) activity, suggesting that EphA2 mediates HGF/SF-induced branching morphogenesis through inhibition of RhoA–ROCK pathway [206]. HGF/SF-induced branching morphogenesis can also be antagonized by several morphogenic factors, such as TGF-β, which inhibit the formation of tubular structures in MDCK cells [202]. Hedgehog signaling in prostate stromal cells downregulates HGF/SF and thus inhibits branching morphogenesis in prostate cells. Such a signaling downregulates HGF/SF expression by inducing miR-26a and miR-26b, which in turn downregulate expression of HGF/SF [207].

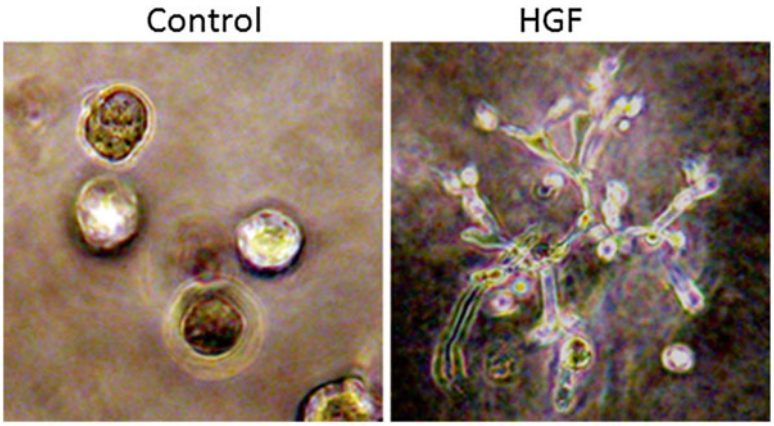

Fig. 8.6 HGF/SF induces branching morphogenesis in DU145 cells. 2,000 cells were suspended in 100 μl medium containing 50 % of Matrigel and loaded into 96-well plates. Cells were cultured in 37 °C for 30 min and fed with normal medium (Control) or medium supplemented with HGF/SF (100 ng/ml) for 10 days (HGF). Cells form acini in the absence of HGF/SF, while a portion of cells form branching structures in the presence of HGF/SF. Shown is a representative picture of branching structures formed when cells were cultured in the presence of HGF (unpublished data provided by Gao)

8.4.4 HGF/SF–MET Signaling and Stem Cell Properties

Stem cells are cells found in multicellular organisms that can divide and differentiate into diverse specialized cell types and can self-renew to produce more stem cells. Cancer stem cells, or cancer-initiating cells, are defined as a subpopulation of cancer cells that effectively reconstitutes the tumor heterogeneity after transplantation [208]. According to stem cell theory, the small fraction of cancer stem cells is the driving force for tumor growth and therefore should be the target of cancer therapy. Cancer stem cell theory has attracted a great interest, although the identity of these cells is still elusive [209].

HGF/SF–MET signaling has been implicated in the migration (but not the proliferation) of human mesenchymal stem cells isolated from bone marrow and cord blood [210, 211], as well as in cardiac stem cells after myocardial infarction [212]. MET signaling has also been implicated in the activation of the adult muscle stem cells [213, 214], hepatic stem cells [215], and pancreas stem or progenitor cells [216], and suggesting it is involved in the regeneration and repair of these organs.

HGF/SF–MET signaling has also been implicated in the stem cell properties of several types of cancers. In colon cancer, myofibroblast-secreted HGF/SF activates β-catenin-dependent transcription and CSC clonogenicity and even restores the CSC phenotype in more-differentiated tumor cells [217]. In human glioma, expression of the *MET* oncogene is associated with a mesenchymal and proneural subtype, but not the classical subtype of glioblastoma. The MET-expressing subpopulation in mesenchymal or proneural subtype neurospheres displays clonogenic potential and long-term self-renewal ability. These stem cell properties are further enhanced by HGF/SF treatment, suggesting that MET is a functional marker of glioblastoma stem cells [218]. A high level of MET is also associated with luminal progenitors in mouse models, and constitutive activation of MET in those progenitors generates stem cell properties, including clonogenic activity and the de novo ability to reconstitute mammary glands in repopulation assays. Activation of MET in luminal progenitors induces hyperplasic ductal morphogenesis and basal lineage commitment. These observations suggest a role for MET in promoting deregulated proliferation and generation of basal-like breast tumors [219].

8.5 HGF/SF–MET in Embryogenesis and Tissue Regeneration

One of the functions of HGF/SF–MET signaling in embryogenesis is in the generation of skeletal muscle that derives from long-range migrating precursor cells. Such precursor cells emigrate from the dermomyotome, an epithelial structure that develops from somites, and finally generate a subset of the hypaxial muscle groups. Loss of the *HGF/SF* or *MET* gene results in complete absence of the hypaxial muscle groups in the mouse embryo, whereas other muscle groups form normally [194, 195].

HGF/SF and MET are also involved in the development of epithelial organs. In *HGF/SF*- and *MET*-null mutant embryos, the liver is reduced in size, and the placental labyrinth layer formed by epithelial trophoblast is greatly reduced [194, 220, 221].

Regeneration is a fundamental part of liver response to injury. Among many growth factors and cytokines, HGF/SF plays important roles in this process [222]. Partial hepatectomy rapidly triggers HGF/SF mobilization from the extracellular matrix and the activation of MET in hepatocytes, which leads to proliferation. Mice with conditional knockout of *MET* in hepatocytes display impaired proliferation and incomplete liver regeneration after partial hepatectomy, providing genetic evidence for the crucial role of MET in liver regeneration [223, 224].

Upon injury to the skin, a set of complex biochemical events takes place in a closely orchestrated cascade to repair the damage. The basal keratinocytes at the wound edges play an important role in the epithelialization stage. HGF/SF and MET are co-expressed in keratinocytes during wound repair of the skin, implying that autocrine signaling is involved [225]. In mice with *MET* knockout in keratinocytes, only cells that had escaped recombination and that continued to express a functional MET could contribute to regeneration [225], suggesting that HGF/SF–MET signaling is essential for re-epithelialization in vivo.

8.6 Role of the MET Receptor Tyrosine Kinase in Human Disease

8.6.1 HGF/SF–MET Signaling in Cancer

MET was originally isolated as an activated oncogene, *Tpr-MET*, which possessed transforming activity [1]. The generation of an autocrine loop by co-expressing wild-type MET and HGF/SF molecules in NIH3T3 cells was also shown to be oncogenic, inducing tumor metastasis [2, 226]. The tumorigenicity of both *Tpr-MET* and autocrine HGF/SF–MET signaling was further proven in transgenic mouse models [227–229].

The involvement of *MET* in human tumorigenesis and metastasis was supported by the detection of *MET* amplification and overexpression in various tumors, especially in metastatic cancers. Most importantly, germline missense mutations in *MET* were discovered in both the sporadic and hereditary forms of human papillary renal carcinomas [230]. Most of these mutations are located in the kinase domain and are homologous to cancer-inducing mutations that occur in other RTKs. These mutants show increased levels of kinase activity, and NIH 3T3 cells expressing mutant MET forms in vitro are tumorigenic in nude mice [231, 232]. Mice carrying these mutations developed a variety of tumors including sarcomas, lymphomas, and carcinomas [233]. When expressed in the mammary gland, the mutant MET molecules induce basal-like breast carcinomas [234, 235]. Somatic MET mutations were detected in childhood hepatocellular carcinomas (HCCs) [236] and head and neck

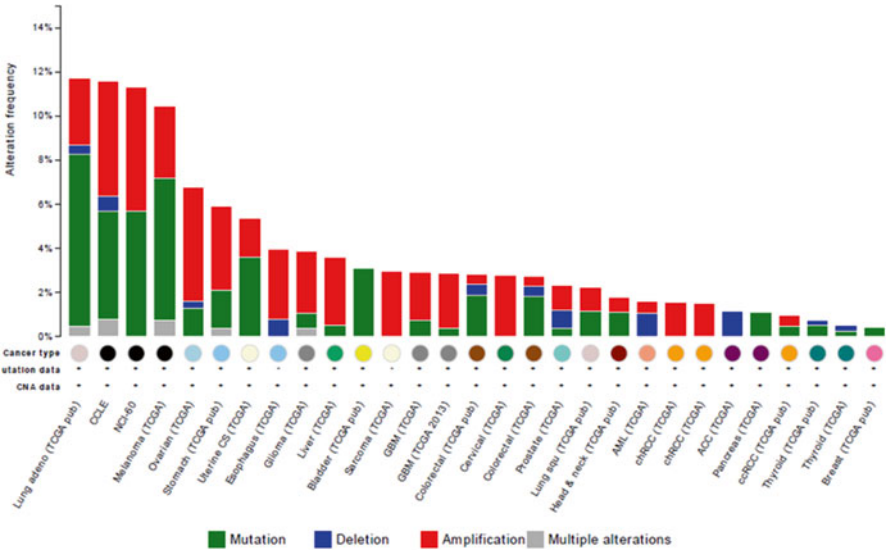

Fig. 8.7 *MET* gene alterations in major human cancer types. Mutations were identified in human cancers in The Cancer Genome Atlas (TCGA). *MET* mutations identified in cancer cell lines in Cancer Cell Line Encyclopedia (CCLE) and NCI-60 Cell Lines (NCI-60) are also included. The diagram is generated by the cBioPortal for Cancer Genomics

squamous cell carcinomas (HNSCC) [237]. The role of MET in tumor metastasis is supported by HNSCC, where the transcripts of the *MET* mutants are highly expressed in lymph node metastases but are barely detectable in the primary tumors, suggesting that the activating mutations of *MET* are clonally selected during the metastasis [236]. Recently, *MET* gene mutations, amplifications, and deletions have been inclusively studied in various types of human cancer in The Cancer Genome Atlas (TCGA) and other cancer genome projects (Fig. 8.7). The genetic alterations distribute across each domain of MET protein (Fig. 8.8).

MET/HGF can be dysregulated in human cancers through a number of other activating mechanisms, such as overexpression and alternative splicing, or HGF ligand-induced autocrine/paracrine loop signaling (refer to http://www.vai.org/met for comprehensive review of HGF/SF and MET in human cancers). For example, hypomethylation of a retrotransposon, LINE-1, was found to induce an alternate transcript of MET in bladder tumors and across the entire urothelium of tumor-bearing bladders [238]. In human breast cancers, deletion of a transcriptional repressor element (DATE, located 750 bp upstream from the transcription start) modulates chromatin structure and DNA–protein interactions, leading to constitutive activation of the *HGF* promoter [35]. Recently a new way for MET signaling to promote tumor metastasis was reported [239]. Highly metastatic melanoma cells produce MET containing exosomes that transfer MET protein to bone marrow progenitors and reprogram the bone marrow cells toward a pro-vasculogenic phenotype. Thus

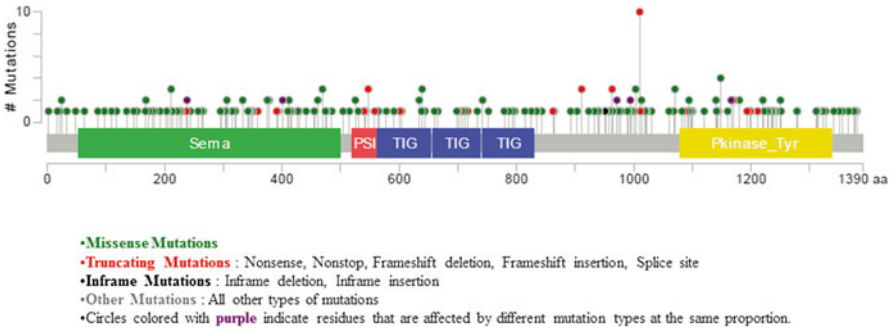

Fig. 8.8 MET mutations identified in human cancers in cancer genome projects. Circles representing mutations are colored according to the mutation type. Where different mutations are found at a single position, the color represents the most frequent mutation type. The diagram was generated by the cBioPortal for Cancer Genomics

melanoma cells increase the metastatic behavior through exosome production, transfer, and education of bone marrow cells to support angiogenesis [239].

It is well established that aberrant MET–HGF/SF signaling contributes to the development and progression of a variety of human cancers, so the interruption of HGF/SF–MET signaling has emerged as a useful intervention strategy. HGF/SF-neutralizing monoclonal antibody mixtures directed against epitopes that block HGF-induced MET signaling markedly inhibit tumor growth in animal models [240]. Subsequently, individual monoclonal antibodies that can block HGF/SF binding to MET have been isolated [241]. Beyond neutralizing antibodies, MET antagonists such as NK1, as well as various types of small molecules that inhibit the MET receptor tyrosine kinase, have been developed [242]. The availability of HGF/SF–MET inhibitors with a range of potencies and specificities has provided a strong basis for assessing their therapeutic value in human cancer, and the initial results from clinical studies have shown therapeutic benefits to patients with a variety of advanced or metastatic tumors, including NSCLC and breast, prostate, liver, and renal cancer. Several therapeutic studies have progressed to Phase III trials. Recently a durable, complete response was reported using an anti-MET receptor monoclonal antibody, MetMAb, in a patient population with chemotherapy-refractory, advanced gastric cancer [243, 244]. However, the cancer recurred after 2 years, and MetMAb therapy achieved a mixed response at recurrence. Larger studies and rigorous patient stratification procedures will clarify the therapeutic value and long-term safety of HGF/SF–MET inhibitors in cancer patients. The development of new intervention strategies that target HGF/SF–MET signaling will finally provide powerful weapon for fighting human cancers.

Drug resistance presents a challenge to target-based cancer therapy. Lung cancer with EGFR-activating mutations that responds initially to the EGFR inhibitors gefitinib and erlotinib invariably develops resistance to them. *MET* amplification has been detected in such lung cancer cell lines and lung cancer specimens.

MET amplification triggers gefitinib resistance through ERBB3-dependent activation of PI3K [134]. In addition to amplification, HGF/SF-mediated MET activation also contributes to the gefitinib resistance in lung cancer [245, 246]. However, in a Phase III lung cancer trial of the MET-specific antibody Onartuzumab in combination with EGFR inhibitor erlotinib did not provide any meaningful benefit over erlotinib alone [247]. The failure may partially due to the unselected population that includes patients with no MET alterations. In Phase II lung cancer trials, Onartuzumab plus erlotinib was associated with improved progression-free survival (PFS) and overall survival (OS) in a prespecified MET-positive population as determined by IHC [248, 249]. Other biomarkers, such as *MET* amplification measured by fluorescence in situ hybridization (FISH), may also be useful in selecting suitable patients [247].

Stromal cell secretion of HGF/SF has been identified as a major factor in the resistance to RAF inhibitors of *BRAF*-mutant melanoma, glioma, and colon cancer cells. In melanoma, the expression of HGF/SF in stromal cells significantly correlates to resistance to RAF inhibitor. Inhibiting HGF/SF or MET results in a reversal of the resistance to RAF inhibitors, suggesting that a combination therapy targeting both RAF and HGF/SF–MET is a therapeutic strategy for *BRAF*-mutant tumors [250, 251].

Vascular endothelial cell growth factor (VEGF) plays a key role in stimulating angiogenesis and driving tumor growth in many forms of cancer. The failure of antiangiogenic therapy with VEGF inhibitors has been partially ascribed to tumor invasion in response to treatment. In a mouse model of glioblastoma multiform (GBM), VEGF enhanced the recruitment of the protein tyrosine phosphatase 1B (PTP1B) to the MET/VEGFR2 complex and suppressed HGF/SF-dependent MET phosphorylation and tumor cell invasion. VEGF blockade with bevacizumab resulted in increases of MET activity and cell invasion. Dual inhibition of VEGF and MET blocked the cell invasion provoked by VEGF and resulted in a substantial survival benefit [252]. Indeed, endothelial cells express high levels of MET, which is activated by HGF/SF produced by tumor cells. The paracrine activation of endothelial MET contributes to tumor angiogenesis and confers resistance to antiangiogenic therapy with sunitinib. A combination of sunitinib and a selective MET inhibitor significantly inhibited tumor angiogenesis [253].

Beyond drug resistance, the activation of MET may also be involved in resistance to ionizing radiation therapy. Radiation induces overexpression and activation of the MET through the ATM-NF-κB signaling pathway in several human tumor cell lines. Activated MET, in turn, protects cells from apoptosis and promotes cell invasion, leading to radioresistance [254].

8.6.2 HGF/SF–MET Pathological Signaling in Diabetes, Autism, and Listeria *Infection*

HGF/SF is a pleiotropic growth factor involved in embryogenesis and in various adult physiological processes. Dysregulation of HGF/SF–MET signaling has been implicated in various diseases in addition to cancer.

The HGF/SF–MET axis regulates metabolism by stimulating hepatic glucose uptake and suppressing hepatic glucose output. MET receptor directly binds to INSR to form a hybrid complex, which is essential for an optimal hepatic insulin response. HGF/SF–MET restores insulin responsiveness in insulin-refractory mice, providing new insights into the molecular basis of hepatic insulin resistance [255]. HGF/SF–MET signaling is also critical for beta-cell survival. Pancreas-specific *MET*-null mice were more susceptible to multiple low-dose streptozotocin (MLDS)-induced diabetes, and they had higher blood glucose levels, marked hypoinsulinemia, and reduced beta-cell mass compared with wild-type littermates. In vitro, *MET*-null beta-cells were more sensitive to cytokine-induced cell death, an effect mediated by NF-κB activation and NO production. These results suggest that the activation of HGF/SF–MET signaling is a potential therapeutic strategy for diabetes [256].

Genetic studies of autism suggest that candidate genes may be located within the chromosome 7q31 region. HGF/SF–MET signaling participates in neocortical and cerebellar growth and maturation, immune function, and gastrointestinal repair, consistent with reported medical complications in some children with autism. A family-based study of autism including 1,231 cases showed a genetic association ($P = 0.0005$) of a common C allele in the promoter region of the *MET* gene in 204 families. Functional assays showed that the C allele results in a twofold decrease in MET promoter activity and in altered binding of specific transcription factor complexes. These data implicate reduced *MET* gene expression in autism susceptibility [257].

The bacterial pathogen *Listeria monocytogenes* uses its surface protein InlB to invade a variety of cell types. The interaction of InlB with MET is crucial for the occurrence of infection. Structural studies have indicated that InlB directly binds to MET to form a 2:2 complex with an InlB dimer at its center and one MET molecule bound peripherally to each InlB [258]. The InlB leucine-rich repeat region interacts with the first immunoglobulin-like domain of the MET stalk. A second contact, between InlB and the MET Sema domain, locks the otherwise flexible receptor in a rigid, signaling-competent conformation [259]. Upon binding of InlB to MET, the ubiquitin ligase Cbl is rapidly recruited to the complex. Purified InlB induces the Cbl-dependent monoubiquitination and endocytosis of MET, and the bacterium exploits the ubiquitin-dependent endocytosis machinery to invade mammalian cells [260, 261].

8.7 Conclusion

HGF/SF–MET signaling plays an important role in embryogenic development and adult physiological processes. Interruption or aberrant activation of HGF/SF–MET signaling has been implicated in several human diseases, especially cancers. Tumor cell addiction to MET and other RTKs is the basis for targeting cancer therapy. However, diverse pathways can be activated in a heterogeneous tumor. Resistant clones supported by signaling that are insensitive to the inhibitor will be selected.

Although combinations of different types of inhibitors may circumvent such resistance, genomic instability and the resulting clonal diversity of tumor cells may present may present a serious challenge for targeting therapy against human cancers. Targeting genomic instability could be the ultimate strategy for effective cancer therapy.

Acknowledgments We thank Kay Koo for administrative support and David Nadziejka for editing the manuscript. This work was supported by the Jay and Betty Van Andel Foundation.

Receptor at glance: comparison between MET and RON

	MET	RON
Other names	HGFR	MST1R; CD136; MSPR; PTK8
Chromosome location	7q31.2	3q21.31
Gene size (bp)	126,193	16,872
Intron/exon	20/21	19/20
mRNA size (5′, ORF, 3′)	6,695	4,785
Amino acids	1,390	1,400
Molecular weight	190	185
Subunit (α-chain/β chain)(kDa)	145/45	150/35
Posttranslational modifications	Proteolytic processing; phosphorylation; ubiquitination	Proteolytic processing; phosphorylation; ubiquitination
Domains	SEMA; CR; IPT; JM; kinase domain; docking site	SEMA; CR; IPT; JM; kinase domain; docking site
Phosphorylation sites	Tyr1234/1235 in kinase domain Tyr1349/1356 in docking site	Tyr1238/1239 in kinase domain Tyr1353/1360 in docking site
Pathways activated	PI3K/AKT2; RAS/MAPK; SRC; STAT3; PLCγ–PKC; Crk	PI3K/AKT2; RAS/MAPK; SRC; STAT3; PLCγ–PKC; Crk; NO
Tissues expressed	Mainly in epithelial cells; also found in endothelial cells, neurons, hepatocytes, hematopoietic cells, and melanocytes	Macrophages; epithelial and keratinocyte cells
Distribution in epithelial cells	Basal lateral membrane	Apical membrane
Transcriptional factor binds to promoter	AP1; SP1; Est1; Pax3; P53; HIF1α	NF-κB; Est-1 and estrogen receptor
Ligand for the receptor	HGF/SF	HGFL/MSP
Cell type that produces ligand	Mesenchymal cells	Hepatocyte
Interaction between ligand and receptor	Paracrine	Endocrine
Induction of cellular responses	Proliferation; scatting; migration/ invasion; surviving; branching morphogenesis; angiogenesis	Proliferation; scatting; migration/ invasion; surviving; branching morphogenesis; angiogenesis

(continued)

	MET	RON
Knockout mouse phenotype	Early embryonic lethality (e13.5)	Early embryonic lethality (e7.5)
Ligand knock out phenotype	Early embryonic lethality (e16.5)	No gross phenotype; fertile
Human diseases	Cancer; autism; diabetes;	Inflammation; cancer
Point mutation in cancers	Papillary renal carcinomas; HCC; lung cancer; brain tumors	Papillary renal carcinomas
Overexpression and aberrant activation	Most types of human cancer	Breast, lung, prostate, gastric, pancreatic, renal, bladder, ovarian, gastrointestinal, and colon cancers

The information about RON was obtained from the review by Wagh et al. [18].

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