Review Article

Biology of MET: a double life between normal tissue repair and tumor progression

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Abstract: MNNG HOS transforming gene (MET) is a class IV receptor tyrosine kinase, expressed on the surface of epithelial cells. The interaction with the hepatocyte grow factor (HGF) induces MET dimerization and the activation of multiple intracellular pathways leading to cell proliferation, anti-apoptosis, morphogenic differentiation, motility, invasion, and angiogenesis. Knock out mice have demonstrated that MET is necessary for normal embryogenesis including the formation of striate muscles, liver and trophoblastic structures. The overexpression of MET and HGF are common in solid tumors and contribute to determine their growth. Indeed, MET has been cloned as a transforming gene from a chemically induced human osteosarcoma cell line and therefore is considered a proto-oncogene. Germline MET mutations are characteristic of hereditary papillary kidney cancers and MET amplification is observed in tumors including lung and gastric adenocarcinomas. The inhibition of MET signaling is the target for specific drugs that are raising exciting expectation for medical treatment of cancer.

Keywords: MNNG HOS transforming gene (MET); oncogene; cancer

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MET structure and its activation

MNNG HOS transforming gene (MET) is a class IV receptor tyrosine kinase, a single pass transmembrane protein with an extracellular domain, a transmembrane hydrophobic sequence and an intracellular portion. The intracellular sequence has tyrosine kinase activity and is necessary for signal transduction (*Figure 1*).

MET is translated as a single chain precursor of 1,390 amino acids (NM_000245). After translation, the protein is transported to the Golgi reticulum and is glycosylated (1). In the post-Golgi compartment, the cellular protease furin cleaves MET precursor between residues 307 and 308, in two chains: alpha (the N-terminal portion) and beta (2). The two chains remain linked by a disulphide-bound forming a heterodimer in the extracellular portion of the receptor (2). The extracellular portion is composed of three domains: a SEMA (semaphorin) domain, a PSI (plexin-semaphorin-integrin) domain and four IPT (immunoglobulin-plexin-transcription) repeats. A large SEMA domain, extended for the first 514 N-terminal residues, includes the whole alpha and part of the beta subunits (308-514 aa) (2). This sequence shares sequence homology with the domains of the semaphorin and plexin families. The structure of the SEMA domain is a seven-bladed beta-propeller and includes the binding site for the hepatocyte grow factor (HGF) (3). PSI domain follows the SEMA and counts about 50 residues with four disulphide bonds. Four IPT domains, which are related to immunoglobulin-like domains, connect the PSI domain with the transmembrane helix. The immunoglobulin-like domains are considered a stalk exposing the SEMA domain to the ligand. The intracellular portion includes the juxtamembrane sequence, the tyrosine kinase catalytic sites and the carboxyl-terminal sequences. The tyrosine kinase activity is increased by the phosphorylation of Tyr1234 and Tyr1235 within the catalytic site and is repressed by that of Ser975 within the juxtamembrane portion (2). Two other tyrosines: Tyr1349 and Tyr1356 belong to the carboxyl-terminal tail and act as a docking

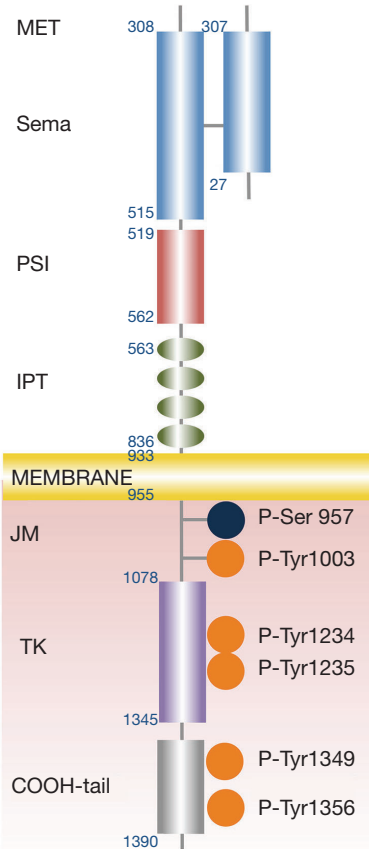

Figure 1 Structure of MET receptor. The extracellular portion of MET is composed by a SEMA domain, a PSI domain and four IPT repeats. The intracellular portion counts a JM a TK domain and a carboxyl terminal tail (COOH-tail). The phosphorylation of serine Ser957 has inhibitory effects whereas the phosphorylation of Tyr1003 is necessary for CBL-binding leading to ubiquitination, internalization and degradation of the receptor. The phosphorylation of Tyr1234 and Tyr1235 of the tyrosine kinase domain occurs with dimerization and is necessary for receptor activation. The phosphorylation of the COOH-tail represents the docking site for adaptor molecules that cooperate to signal transduction. The NM_000245.2 transcript was used as reference for the positions. SEMA, semaphoring; PSI, plexin-semaphorin-integrin; IPT, immunoglobulin-plexin-transcription; JM, juxtamembrane; TK, tyrosine kinase.

site for the recruitment of multiple transducers and adaptors (2).

Gene and transcripts

MET locus is mapped on chromosome 7q21-31 (chr7: 116312411-116438440 according to NCBI 37) and counts 21 exons. The entire first exon is not translated (5'-UTR) as well as the first 14 nucleotides of exon 2 after which the coding sequence begins. Exon 13 contains the sequence that encodes for the hydrophobic transmembrane domain. Therefore, exons 14-21 represent the intracellular domains (*Table 1*). At least three different transcript isoforms of MET have been described originating from alternative splicing (4). The most commonly expressed isoform in human tissue and cell lines (NM_000245.2) lacks of 54 nucleotides of exon 10 (5). Alternative splicing of exon 14 generates another isoform with an in-frame deletion of 47 amino acids in the juxtamembrane domain, which lacks of the Tyr1003 necessary for CBL binding and protein degradation (6). This isoform has been associated to pathological process such as cancer growth because of the reduced receptor internalization and degradation (7). MET promoter lacks TATA or CAAT elements and contains four putative binding sites for the transcription factors ETS, indeed ETS1 induces *MET* transcription *in vitro* (2,4). Moreover, *MET* promoter has hypoxia response elements (HREs) that can bind to HIF1 during hypoxic conditions (2).

Table 1 Exon-domain relationship

Exons	Domains
Exon 2	Cleavage site for alpha and beta subunits
Exon 2-13	Extracellular domain
Exon 13	Hydrophobic transmembrane domain
Exon 13-21	Intracellular domain
Exon 15-21	Kinase domain
Exon 16	ATP binding site
Exon 19	Autophosphorylation site
Exon 21	SH domain

Intracellular MET signaling

MET has been observed on the cell surface in monomer and dimers (8). HGF induces homo-dimerization and phosphorylation of two tyrosine residues (Tyr1234 and Tyr1235) of the catalytic loop of the kinase domain. Subsequently, the tyrosine residues Tyr1349 and Tyr1356 of the carboxy-terminal tail become phosphorylated forming a tandem SH2 recognition motif (9) that is able to recruit several signaling effectors including GRB2, SHC, CRK, PI3K, PLCγ, SRC, SHIP2 and STAT3 (10).

Gab1 knock out embryos show the same defects of those

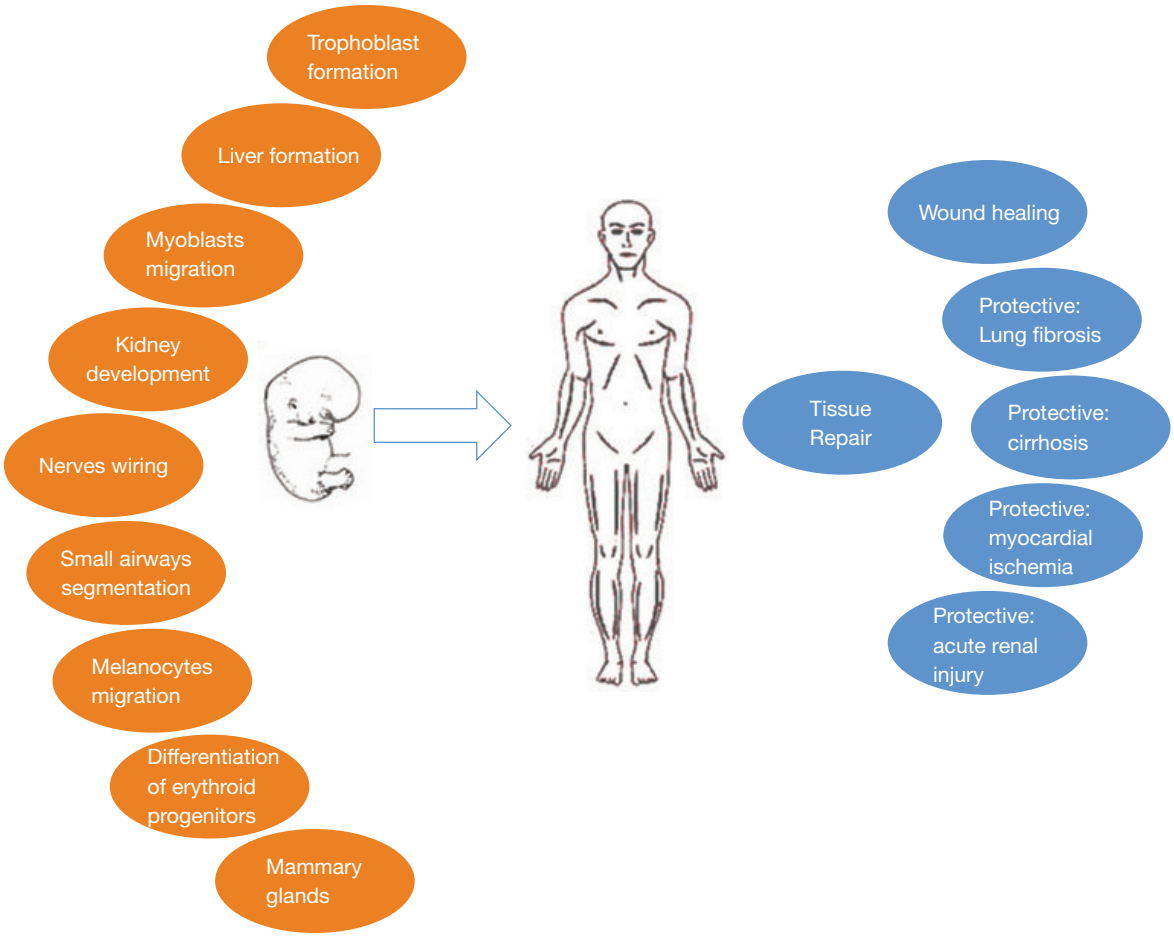

Figure 2 Summary of biological function of HGF-MET signaling during embryogenesis (on the left side) and in adult life (on the right side). HGF, hepatocyte grow factor.

MET or HGF null (11,12). The GAB1 adaptor protein can bind directly to phosphorylated MET or through GAB2 and creates the binding site for more downstream adaptors (1). The direct interaction requires 13 amino acids of GAB1 that constitute the binding site for MET and interact directly with Tyr1349 of the carboxyl-terminal tail (13). This unique interaction sustains activation of several signaling pathways recruited by GAB1. The indirect interaction with GRB2 not only recruits GAB1 but also is necessary for KRAS activation leading to tumorigenesis and metastatic spread (14,15).

The complete plethora of downstream signaling of MET has not been fully elucidated; different pathways can be observed in different tissues (2). However, large-scale phospoproteomics studies demonstrated highly conserved core elements of MET signaling (16-18). In the presence of oncogene addiction, specific inhibitors determine dephosphorylation of elements belonging to similar intracellular pathway in EGFR mutant and MET amplified cells (18).

Indeed, the activation of MET has been linked to the best-known intracellular signaling pathways (*Figure 2*). Through the activation of MAPK pathway, MET can stimulate cell proliferation, cell cycle progression and cell mobility (19,20). The activation of KRAS and the downstream MAPK pathway occurs through the binding of SHC and GRB2 to the activated MET (21).

MET can promote cell survival through PI3K-AKT pathway. The p85 subunit of PI3K can bind the activated MET directly or indirectly through GAB1 (22). The activation PI3K-AKT pathway induces the transcription of the anti-apoptotic proteins BCL2 and BCL-XL that sustain the pro-survival signal (23). Together with SRC, PI3K-AKT is an intermediary for the MET dependent activation of NF-κB (24).

STAT3 can directly bind to MET and after

phosphorylation can migrate to the nucleus to induce tubulogenesis (25) and invasion (26).

The activation of MET-SRC-FAK axis leads to cell migration and promotion of anchorage independent cell grow (27,28). Moreover, SRC activation induces a positive feedback on MET activation (16,27).

HGF exerts pro-angiogenic properties promoting the formation of blood vessels (29) through the induction of the vascular endothelial growth factor (VEGF) and the inhibition of thrombospondin, a negative regulator of angiogenesis (30,31).

Several phosphatases negatively regulate MET receptor including LAR that inhibits MET's activation and induces contact inhibition of cell growth. Moreover, DEP1 (32), PTP1B, and PTP1B phosphatases regulate phosphorylation of MET (33). Negative regulation of MET signaling occurs by phosphorylation of tyrosine Tyr1003 in the juxtamembrane domain (2). The phosphorylation of tyrosine Tyr1003 in the juxtamembrane domain is necessary for CBL E3-ligase binding through SH2-like TKB domain of the CBL (34). CBL binding determines ligand-dependent ubiquitination of MET, internalization of the receptor and subsequently its lysosomal degradation (2). MET Tyr1003 mutants cannot bind to CBL and result in an enhanced stability of MET that is responsible for transformation and tumorigenic properties (2,35). MET degradation can occur also in an ubiquitin independent manner that does not require the kinase activity of the receptor. The disintegrine metalloprotease ADAM cleaves the extracellular NH terminal portion of MET (receptor shedding). Whereas membrane anchored cytoplasmic tail undergoes proteasome degradation, the extracellular domain is released and can sequester HGF or bind the full length MET preventing its activation (36). The treatment with monoclonal antibodies anti-MET increases the shedding of the receptor (36).

Beside the classical activation due to HGF, MET can interact with several cell surface proteins including semaphorin (37), beta-4 integrin (38), and CD44 (39). The interaction between MET and beta-4 integrin determines invasive growth (38), whereas that with CD44 links MET to actin and cytoskeleton (39). Because of the similarities in the extracellular SEMA-domain some semaphorines can activate MET in absence of HGF inducing MET dependent biological responses such as invasion (36). A putative MET-HER2 interaction induces loss of epithelial polarity and enhances invasion, in three-dimensional epithelial cell cultures (40).

Biological function of HGF-MET signaling

MET is present on the surface of epithelial cells of multiple organs including liver, pancreas, prostate, kidney, lung and bronchus (41,42). HGF, also known as scatter factor, is secreted by mesenchymal cells as a single chain precursor that becomes active after the cleavage by extracellular proteases into alpha and beta subunits. These subunits remain linked by a disulphide bound. *In vitro*, HGF is a potent inductor of proliferation for primary culture of hepatocytes and renal tubule cells and stimulates cell dissociation and sprouting (43).

In vivo, HGF is a pleiotropic factor that stimulates proliferation, survival, motility, scattering and differentiation (*Figure 3*) (2). For example, HGF plays a direct role in proliferation and differentiation of erythroid progenitors (44). The paracrine loop between HGF secreted by mesenchymal cells and its receptor MET, present on epithelial and endothelial cells, promotes the migration and proliferation of stem cells that is necessary for tissue repair and wound healing (45-48).

HGF exerts a protective role in lung fibrosis (49) and liver cirrhosis (50). The activation of HGF-MET signaling in cells of kidney's tubules exerts a protective effect after acute injury (51). Similarly, the activation of MET protects myocardiocytes during revascularization after ischemia (48). During tissue repair, several cytokines, present in the interstitial compartment including IL-1, IL-6, TNF-α and TGF-β, induce transcription of HGF and of MET in fibroblast-macrophages and in epithelial cells, respectively. Therefore, HGF becomes abundant and activated in the interstitial compartment during inflammation. This leads to MET activation as part of the physiological defense to tissue damage (36).

HGF induces cell scattering: a phenomena characterized by the lost of cell-cell contacts mediated by cadherin: allowing the cells to migrate (52). Madine–Darby cell line responds to HGF stimulation by scattering in two-dimensional cultures and forming tubules in three-dimensional cultures. These are key epithelial functions in wound repair and embryogenesis (53).

MET is necessary for the normal development of muscles because affects the migration of muscular cell precursors and for formation of the liver and the placenta since provides proliferation and survival signals for hepatocytes and trophoblastic cells (54,55). In c-Met homozygous mutant (–/–) mouse embryos, the skeletal muscles of the limb and diaphragm do not form because of the

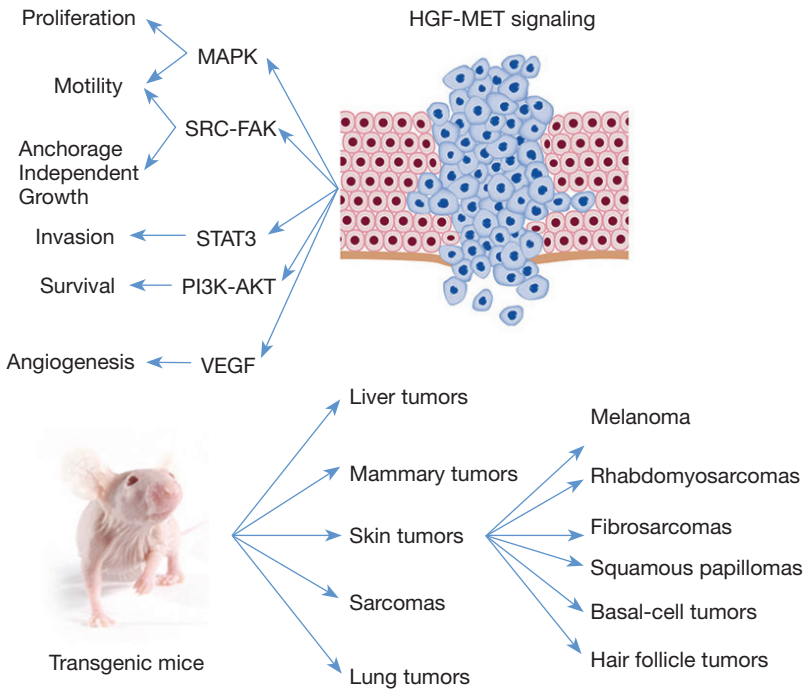

Figure 3 Summary of HGF-MET signaling in cancer. Evidences derived from *in vitro* (upper panel) and *in vivo* experiences (lower panel). HGF, hepatocyte grow factor; VEGF, vascular endothelial growth factor.

myogenic precursor cells do not colonize the limb bud and diaphragm. In contrast, the axial skeletal muscles present a normal development in the absence of c-Met signaling (55). Mice lacking HGF fail to complete development and die in utero. The mutation affects the embryonic liver, which is reduced in size and shows extensive loss of parenchymal cells. In addition, development of the placenta, particularly of trophoblast cells, is impaired resulting in a hypomorphic organ that causes in utero lethality (56,57). Transgenic mice lacking EGFR and MET signaling present smaller kidneys and a reduced number of nephrons (58).

On the contrary, transgenic mice with HGF under the control of metallothionein promoter present an increased size of their liver: about a double ratio of liver/body weight (59). A dramatic increase of 2N small hepatocytes is observed in transgenic livers. Hepatocytes isolated by perfusion of transgenic livers show a doubling time of 2 days in culture compared to no growth of wild type ones (59). This proliferation is sustained by chronic activation of MET and its downstream pathways. Indeed, transgenic mice have a much faster liver regeneration than controls after partial hepatectomy (59). Transgenic mice expressing HGF under the albumin promoter present lower level of HGF compared to those with HGF under the metallothionein promoter and a milder increase of liver size (60). Moreover, transgenic mice with HGF under the control of metallothionein promoter exhibit ectopic skeletal muscles and melanocytes in the central nervous system (61). Finally, the disruption of HGF-MET signaling alters the formation of nervous system connections with a reduced survival of sensory and sympathetic neurons and reduced outgrowth of some motor nerves (62-64).

MET in human cancers

HGF-MET signal promotes detachment of normal cells, without the activation of anoikis allowing their migration. This is necessary for the formation of several organs during embryogenesis and in adult life for tissue repair and wound healing. Cancer cells resume physiological programs, normally activated during embryogenesis, to achieve and enhance invasiveness and metastatic spread.

An altered form of MET (TPR-MET), of 65KDa, with constitutive kinase activity, initially named MNNG-HOS, has been cloned as a transforming factor from a chemically induced human osteosarcoma cell line and therefore, MET is considered a proto-oncogene (65). The cloned transcript is able to transform NIH-3T3 cells *in vitro*. The fusion

protein originated from the translocation t(1q25;7q31) juxtaposes TPR (translocated promoter region) and the intracellular domain of MET (66,67). The fusion protein forms dimers through the leucine zipper encoded by the TPR portion resulting in a constitutively activated MET even in the absence of its ligand (68).

MET overexpression is observed in many human tumors (www.vai.org/HgfSf-MET and cancer), which is, frequently, associated with a metastatic phenotype and poor prognosis (43). In colorectal cancer, MET amplification and expression correlates with a more advanced stage, tumor invasiveness and presence of metastases both in lymph node and liver (69,70). Similar results have been reported in ovarian (71-73) and breast cancers (74). In non-small cell lung cancer (NSCLC), MET is overexpressed in 25-75% of the cases and is associated with poor prognosis (75-81). The contextual overexpression of HGF can generate a paracrine loop that possibly sustains the cancer growth such as demonstrated in transgenic animals (82). Amplification of MET has been described in a minority of NSCLCs (1.4-7%) (83-85) and in gastric (86), esophageal (87), colorectal (88) and clear cell ovarian cancer (89).

Germline MET mutations have been observed in patients with hereditary papillary renal cell carcinomas (HPRCC) providing the first secure evidence of a link between MET and human oncogenesis (90). Papillary renal cell carcinoma accounts for 10-15% of primary kidney tumors. A familial syndrome, associated with germline mutations of MET, is responsible for a minority of the cases and induces tumors with an incomplete penetrance. These mutations interest the tyrosine kinase domain of MET (90), are able to induce auto phosphorylation of the receptor and to transform NIH 3T3 cells *in vitro* (*Table 2*) (90). Mutant and wild type MET have been transfected in NIH-3T3 cells. The mutant isoforms more potently induce tumor formation after injection in nude mice (110). Only 13% of the sporadic papillary renal cell carcinomas present MET mutations (103). In these tumors, germline and somatic MET mutations have been described (103). Somatic MET mutations are more tumorigenic because induce more transformation of NIH-3T3 cells *in vitro* and xenograft proliferation *in vivo* (110). This suggests a negative selection of the more potent activating mutations during embryogenesis and development (110). Moreover, the germline mutations, observed in sporadic papillary renal cell carcinomas, show tumorigenic activity. This implies a *de novo* mutation; a different occurrence than single nucleotide polymorphisms not related to cancer.

Moreover, somatic mutations of MET tyrosine kinase domain have been described in head and neck squamous cell carcinomas (Tyr1248C and Tyr1253) (71), in a glioma (Gly1137Val) (107) and in a mucinous ovarian carcinoma (Ala1209Gly) (109). In childhood hepatocellular carcinomas, mutations affected both the tyrosine kinase domain (Thr1191Ile) and the carboxyl-terminal tail (Lys1262Arg and Met1268Ile) (108).

More difficult is the interpretation of mutations reported exclusively in tumor DNA without knowing the status of patients' normal DNA. This is the case of several reports regarding NSCLCs, small cell lung cancers (SCLCs) and mesotheliomas (*Table 2*). In these tumors mutations in the SEMA and juxtamembrane domains have been reported (7,91,96,97,99,100). It is difficult to understand their impact on cancer growth in the absence of a wild type allele in normal DNA or outside the context of a familiar cancer related syndrome. Recently, data from the 1,000 genome project have become available showing that some of the putative MET mutations are also present in normal subjects and possibly polymorphisms. These mutations include Thr1010Ile, Arg988Cys and Asn375Ser (111). Different consideration should be made for the somatic mutation observed in NSCLC that determines the skipping of exon 14 (97). Exon 14 contains the tyrosine Tyr1003 which phosphorylation allows CBL binding with subsequent internalization and disruption of the receptor (112).

Recently, high throughput molecular evaluations of lung cancers have demonstrated somatic missense mutations in SCLC (113) and squamous cell carcinomas (*Table 3*) (114). Interestingly, in adenocarcinomas of the lung, MET locus was included in a peak of amplification according to GISTIC analysis strongly indicating its relevance for the growth of these tumors (118). Moreover, MET was significantly mutated according to MutSig2CV algorithm (*Table 3*). RNA sequencing demonstrated MET exon 14 skipping in 4% of cases (118). Data indicate the relevance of MET genomic aberrations for the growth of lung adenocarcinomas and suggest that MET could represent a target for therapy. Finally, MET amplification has been described such as a cause of acquired resistance during treatment with tyrosine kinase inhibitors in patients with EGFR mutations (83,119).

In vivo models of HGF-MET driven tumors

Coexpression of wild type MET and HGF in the same NIH 3T3 cell generates an autocrine loop that increases

Table 2 MET mutations

Mutation	Germline/somatic	Domain	Cancer type	1,000 Genome project	Functional tests	Provean	SIFT	Interpretation of effect on cancer growth	COSMIC
V136I	–	SEMA	RRC (91)	rs199701987	–	Neutral	Tolerated	Weak	
E168D	Germline	SEMA	NSCLC (91)	rs55985569	Possibly tumorigenic	Neutral	Tolerated	Weak	COSM706
	–		NSCLC (7)						
	Somatic		SCLC (92)						
	–		HNSCC (93)						
L229F	Somatic	SEMA	NSCLC (7)	–	–	Neutral	Tolerated	Weak	COSM13244
T230M	–	SEMA	HNSCC (93)	rs200138253	–	Neutral	Damaging	Weak	–
S323G	Germline	SEMA	NSCLC (7)	–	–	Neutral	Tolerated	Weak	–
N375S	Germline	SEMA	NSCLC (94,95)	rs33917957	Possibly tumorigenic	Neutral	Tolerated	Weak	COSM710
	–		NSCLC (91)						
	–		Mesothelioma (96)						
	–		CRC (97)						
	–		SCLC (97)						
	–		HNSCC (93)						
	–		Melanoma (91)						
M431V	–		Mesothelioma (96)	–	–	Neutral	Tolerated	Weak	–
N454I	–		Mesothelioma (96)	–	–	Deleter	Damaging	Weak	–
N930S	–	JM	Melanoma (98)	–	–	Neutral	Tolerated	Weak	–
G965V		JM	Melanoma (98)	–	–	Neutral	Damaging	Weak	–
Q960L	–	JM	RCC (91)	–	–	Neutral	Damaging	Weak	–
R970C	–	JM	SCLC (92,99)	rs34589476	Contradictory	Neutral	Damaging	Weak	COSM1447461
	Germline		NSCLC (7)						
	–		HNSCC (93)						
	–		NSCLC (100)						
	–		Melanoma (98)						
	Germline		Cancer and normal (101)						
	Germline		SCLC (97)						
P978S	–	JM	SCLC (99)	–	–	Deleter	Damaging	Weak	COSM1666979
R982S	–		Melanoma (98)	–	–	Neutral	Damaging	Weak	–
P991S	Germline	JM	Gastric cancer (102)	–	Possibly tumorigenic	Neutral	Damaging	Weak	–

Table 2 (*continue*)

Table 2 (*continue*)

Mutation	Germline/somatic	Domain	Cancer type	1,000 Genome project	Functional tests	Provean	SIFT	Interpretation of effect on cancer growth	COSMIC
T992I	–	JM	Mesothelioma (96)	rs56391007	Contradictory	Neutral	Damaging	Weak	COSM707
	Germline		Breast Cancer (102)						
			NSCLC (100,102)						
	Somatic		NSCLC (92)						
	Germline		HNSCC (93)						
	–		SCLC (99)						
	Germline		PRCC (103)						
	Germline		NSCLC (7)						
ex14sk	Somatic	JM	NSCLC (7)	–	Tumorigenic	Splicing	Splicing	Strong	COSM13245
V1092I	Germline	TK	HPRCC (104)		Tumorigenic	Neutral	Damaging	Strong	–
	Germline		PRCC (105)						
H1094L	Somatic	TK	PRCC (103)		Tumorigenic	Deleter	Damaging	Strong	COSM698
H1094Y	Somatic	TK	PRCC (103)		Tumorigenic	Deleter	Damaging	Strong	COSM696
H1094R	Somatic	TK	PRCC (103)		–	Deleter	Damaging	Strong	COSM703
N1000Y	–	TK	MTS (106)		Possibly tumorigenic	Neutral	Tolerated	Moderate	COSM702
H1106D	Somatic	TK	PRCC (103)		Tumorigenic	Deleter	Damaging	Strong	COSM697
G1119V	Somatic	TK	Glioma (107)	–	–	Neutral	Damaging	Strong	COSM692
M1131T	Germline	TK	HPRCC (90)		Tumorigenic	Deleter	Damaging	Strong	COSM704
T1173I	Somatic	TK	Childhood HCC (108)		–	Neutral	Damaging	Strong	COSM693
V1188L	Germline	TK	HPRCC (90)		Possibly tumorigenic	Deleter	Damaging	Moderate	–
G1191A	Somatic	TK	Ovarian Cancer (109)	–	–	Deleter	Damaging	Moderate	COSM712
L1195V	Germline	TK	HPRCC (90)		Tumorigenic	Deleter	Damaging	Strong	COSM688
V1220I	Germline	TK	HPRCC (90)		Possibly tumorigenic	Neutral	Damaging	Moderate	COSM1673476
D1228H	Somatic	TK	PRCC (90)		Tumorigenic	Deleter	Damaging	Strong	COSM689
D1228N	Germline	TK	HPRCC (90)		Tumorigenic	Deleter	Damaging	Strong	–
Y1230H	Somatic	TK	PRCC (90)		Tumorigenic	Deleter	Damaging	Strong	COSM690
Y1230C	Germline	TK	HPRCC (90)		Tumorigenic	Deleter	Damaging	Strong	COSM699
Y1230D	Germline	TK	PRCC (103)		Tumorigenic	Deleter	Damaging	Strong	–
Y1235D	Somatic	TK	HNSSC (71)		Possibly tumorigenic	Deleter	Damaging	Strong	COSM700
K1244R	Somatic	TK	Childhood HCC (108)		–	Neutral	Tolerated	Strong	COSM695

Table 2 (*continued*)

Table 2 (*continue*)

Mutation	Germline/somatic	Domain	Cancer type	1,000 Genome project	Functional tests	Provean	SIFT	Interpretation of effect on cancer growth	COSMIC
M1250T	Somatic	TK	PRCC (90)	–	Tumorigenic	Deleter	Damaging	Strong	COSM691
M1250I	Somatic	TK	Childhood HCC (108)	–	–	Deleter	Damaging	Strong	COSM694
T1257I	–	TK	HNSCC (93)	–	–	Deleter.	Damaging	Weak	–
V1315I	–	TK	HNSCC (93)	–	–	Neutral	Tolerated	Weak	–

Mutation position was expressed according to NM_000245.2 transcript. Ex14sk denotes a mutation that determines exon 14 skipping, respectively. Germline/somatic reports if the mutation was germline (in tumor and normal DNA), somatic (in tumor but not in normal DNA) or was identified only in tumor DNA but data were not available for patient's normal DNA (-). Domain indicates if the mutation belongs to the SEMA domain, to the JM, or to the TK. Cancer type explains the histotype of the cancer harboring the mutations together with its references. 1,000 Genome project indicates if the mutation has been reported such as a polymorphism in that database. Functional test reports a summary of literature regarding the tumorigenic effect of the described mutations. Provean and SIFT predictions indicate the possible effect of the mutation on the structure of the protein (http://provean.jcvi.org/genome_submit_2.php). Interpretation of effect on cancer growth depends on mutations characteristics and has been graded in weak, moderate or strong association. COSMIC indicates the presence of the mutation in that tumor mutation database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/). SEMA, semaphoring; JM, juxtamembrane; TK, tyrosine kinase; RRC, renal cell carcinoma; NSCLC, non-small cell lung cancer; SCLC small cell lung cancer; HNSCC, head and neck squamous cell carcinoma; CRC, colorectal cancer; PRCC, papillary renal cell carcinoma; HPRCC, hereditary papillary renal cell carcinoma; MTS, metastasis, HCC, hepatocellular carcinoma; delete, deleterious.

Table 3 MET mutations in lung cancer detected using whole and exome sequencing

Mutation	Germline/somatic	Domain	Cancer type	1,000 Genome project	Functional tests	Provean	SIFT	Interpretation of effect on cancer growth	COSMIC
I565M	Somatic	–	SCC (114)	–	–	Neutral	Damaging	Weak	COSM744047
T698S	Somatic	IPT	SCC (114)	–	–	Neutral	Tolerated	Weak	COSM744046
T1096S	Somatic	JM	SCC (114)	–	–	Neutral	Tolerated	Weak	COSM744042
I941M	Somatic	TM	SCLC (113)	–	–	Neutral	Damaging	Weak	COSM317623
Ex3sk.	Somatic	SEMA	SCLC (115)			Splicing	Splicing		
K2N	Somatic	–	AC (116)	–	–	Neutral	Damaging	Moderate	COSM370244
C98F	Somatic	SEMA	AC	–	–	Deleter	Damaging	Strong	COSM598588
Q99L	Somatic	SEMA	NSCLC	–	–	Neutral	Tolerated	Weak	–
Y321H	Somatic	SEMA	AC	–	–	Neutral	Tolerated	Moderate	COSM1548084
N315S	Somatic	SEMA	AC	–	–	Deleter	Damaging	Strong	COSM598585
S441C	Somatic	SEMA	AC	–	–	Deleter	Damaging	Strong	COSM1548082

Table 3 (*continued*)

Table 3 (*continue*)

Mutation	Germline/somatic	Domain	Cancer type	1,000 Genome project	Functional tests	Provean	SIFT	Interpretation of effect on cancer growth	COSMIC
H644N	Somatic	IPT	AC (116)	–	–	Neutral	Tolerated	Moderate	COSM353469
T660R	Somatic	IPT	AC	–	–	Deleter	Tolerated	Moderate	COSM1548081
P742A	Somatic	IPT	AC (116)	–	–	Deleter	Damaging	Strong	COSM375467
Y1003F	Somatic	JM	AC (116)	–	Tumorigenic	Deleter	Damaging	Strong	COSM339515
Y1003N	Somatic	JM	NSCLC (117)	–	Tumorigenic	Deleter	Damaging	Strong	COSM48564
Y1003*	Somatic	JM	AC (118)	–	–	Nonsense	Nonsense	Moderate	COSM598583
Ex14sk	Somatic	JM	AC (118)	–	–	Splicing	Splicing	Strong	COSM24687
Ex14sk	Somatic	JM	AC (118)	–	–	Splicing	Splicing	Strong	COSM35468
Ex14sk	Somatic	JM	AC (118)	–	–	Splicing	Splicing	Strong	–
Ex14sk	Somatic	JM	AC (118)	–	–	Splicing	Splicing	Strong	COSM29633
Ex14sk	Somatic	JM	AC (118)	–	–	Splicing	Splicing	Strong	–
Ex14sk	Somatic	JM	AC (118)	–	–	Splicing	Splicing	Strong	–
H1068L	Somatic	JM	AC (116)	–	–	Deleter	Damaging	Strong	COSM376353
M1160L	Somatic	TK	AC (116)	–	–	Deleter	Damaging	Strong	COSM360346
K1193fs	Somatic	TK	AC (116)	–	–	Frameshift	Frameshift	Weak	COSM391284
Y1235D	Somatic	TK	AC (118)	–	Tumorigenic	Deleter	Damaging	Strong	COSM700
E1314G	Somatic	TK	AC (118)	–	–	Neutral	Damaging	Strong	COSM598580
F1341L	Somatic	TK	AC (116)	–	–	Neutral	Tolerated	Moderate	COSM404626

Mutation position was expressed according to NM_000245.2 transcript. Ex3sk and ex14sk denote mutations that determine exon 3 and 14 skipping, respectively. Germline/somatic reports if the mutation was germline (in tumor and normal DNA), somatic (in tumor but not in normal DNA) or was identified only in tumor DNA but data were not available for patient's normal DNA (–). Domain indicates if the mutation belongs to the SEMA domain, to the IPT domain, to the TM, to the JM, or to the TK domain. Cancer type explains the histotype of the cancer harboring the mutations together with its references. 1,000 Genome project indicates if the mutation has been reported such as a polymorphism in that database. Functional test reports a summary of literature regarding the tumorigenic effect of the described mutations. Provean and SIFT predictions indicate the possible effect of the mutation on the structure of the protein (http://provean.jcvi.org/genome_submit_2.php). Interpretation of effect on cancer growth depends on mutations characteristics and has been graded in weak, moderate or strong association. COSMIC indicates the presence of the mutation in that tumor mutation database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/). IPT, immunoglobulin–plexin–transcription; TM, transmembrane portion; JM, juxtamembrane; SEMA, semaphoring; TK, tyrosine kinase; RRC, renal cell carcinoma NSCLC, non–small cell lung cancer; SCLC, small cell lung cancer; SCC, squamous cell carcinoma; AC, adenocarcinoma; delete, deleterious.

tumor formation and metastatic dissemination when cells are implanted in nude mice (120). On contrary, uncleavable form of HGF binds with high affinity MET and inhibits its activation (121). The local expression of uncleavable HGF within xenograft suppresses tumor growth, impairs tumor angiogenesis, and prevents metastatic dissemination; whereas the systemic expression of uncleavable HGF dramatically inhibits the growth of transplanted tumors and abolishes the formation of spontaneous metastases (121).

HGF-MET pathway is relevant for the growth of lung tumors. When recombinant HGF was injected within SCID mice's xenograft of lung adenocarcinoma cells, tumor showed a 3-fold larger volume than saline-injected controls (122).

Transgenic mice with HGF under control of metallothionein promoter show an increased rate formation of hepatocellular carcinomas and adenomas. Tumors are developed with a long latency because observed only after 17 months of age in FVB/N strain (59). Tumors originate from both epithelial and mesenchymal cells in a wide variety of tissues. Most frequently, tumors arise in the female mammary gland but also skin tumors are common, including melanoma, rhabdomyosarcomas, fibrosarcomas, squamous papillomas, basal-cell and hair follicle tumors. Met phosphorylation is observed in these tumors, suggesting that autocrine signaling broadly promotes tumorigenesis (123). In the same model, HGF overexpression promotes hepatocarcinogenesis and VEGF induces angiogenesis after treatment with diethylnitrosamine, a well-characterized genetic mutagen (124). However, contradictory results have been reported, Shiota *et al.* describe that the overexpression of the human full-length HGF isoform under the transcriptional regulation of the albumin promoter in mice (FVB genetic background) do not induce hepatocellular carcinoma development (60). Moreover, the cross breading of *Hgf* and *Myc* transgenic mice determines a dramatic inhibition of hepatocarcinogenesis in the hybrids (125).

The transgenic mice, overexpressing HGF under metallothionein promoter, present an inappropriate abundance of melanocytes in the dermis, dermo-epithelial junction and in basal layer of the skin (61); an increased number of melanomas are present in these animals (126). However, there is a long latency before these mice develop melanomas. The treatment with carcinogenetic agents (7,12-dimethylbenz[a]anthracene and 12-O-tetradecanoylphorbol-13-acetate) strongly increases the number and the formation of metastatic melanomas in transgenic mice that overexpress HGF (127). The gain of function mutation of the cell cycle control cyclin (CDK4) and the deletion of its inhibitor CDKN2A (p19INK) are key genetic events frequently observed in melanomas (128,129). Transgenic mice with HGF overexpression that bear $CDK4^{Arg24Cys}$ mutation present a dramatic increase in the number of carcinogen-induced primary melanomas of the skin and their metastasis (127).

Similar results are observed in mice overexpressing met and its oncogenic isoforms. Transgenic mice overexpressing met under the control of metallothionein 1 promoter present severe breading defects. Two mammary adenocarcinomas have been observed in met overexpressing mice with Met1268Thr and Tyr1248His mutations (130). When the expression of met is limited to hepatocytes and posed under the control of doxycycline, transgenic mice develop in sequence hyperplastic foci, dysplastic liver foci, and overt tumors either hepatocellular carcinomas or hepatocellular adenomas by 3 months of age (131). Hepatocellular carcinomas do not develop within hepatocellular adenomas and vice versa. Whereas all the hepatocytes expressed transgenic MET, only those of neoplastic and dysplastic foci expressed an activated receptor (phosphorylation of Tyr1234 and Tyr1235) (131). The development of hepatocellular carcinomas or hepatocellular adenomas depends on the occurrence of additional genetic events: activation of beta-catenin or inactivation of the HNF1α pathway, respectively (131). The inactivation of the transgenic met by administration of doxycycline leads to regression of hepatocellular carcinomas in the animals (132) supporting the idea that MET could be an effective target for therapy despite the presence of additional mutations in the same tumor.

Truncation of MET extracellular domain generates a constitutively activated tyrosine kinase: the intracellular portion of MET (cytoMET) is a weak transforming factor (133). Transgenic mice expressing cytoMET in the liver under the control of α1-antitripsine have been generated. These mice do not develop liver tumors during their life and are more resistant to pro-apoptotic stimuli (activation of FAS receptor through JO2 antibody). Hepatocyte cell lines can be established from their liver and maintain a differentiate phenotype and do not undergo transformation (134). Human MET is not activated by murine HGF (135,136). Four lines of transgenic mice have been created in order to express human MET in murine hepatocytes in absence of doxycycline (132). Mice of lines 1 and 2 were born with fatty liver and died within 2 months from the partum. If feed with doxycycline during the pregnancy and for 4 weeks after partum, mice grew normally until 10 months when started to die; 85% of these mice developed hepatocellular

carcinomas. Mice of lines 3 and 4 were healthy at birth but started to die after 4 months: about 85% of them for hepatocellular carcinomas (132). Mice firstly developed foci of hyperplasia that then progressed to malignancy (hepatocellular carcinomas). Treating mice bearding hepatocellular carcinomas with doxycycline, to inhibit MET expression, determined the regression of the nodules (132). Authors showed that the overexpression of MET in presence of cell adherence was sufficient to activate MET signaling since none of the oncogenic mutations of MET were present and murine HGF was ineffective to activate MET signaling (132).

Transgenic mice expressing human oncogenic protein TRP-MET under the control of metallothionein 1 promoter mainly develop mammary gland tumors (137).

Met mutations have been knocked-in in four C57BL/6 mouse lines harboring: Asp1226Asn, Tyr1228Cys, Met1248Thr and the double mutation Met1248Thr/Lys1193Val (138). The mutated forms of *MET* were expressed under the control of the endogenous Met promoter. Despite the strong activity of these mutations *in vitro*, an unexpected long latency before tumor formation is observed (138). Different mutations induce different type of tumors: MET1248Thr($^{mu/+}$) develops only carcinomas, whereas Asp1226Asn($^{mu/mu}$), Tyr1228Cys($^{mu/+}$), and MET1248Thr/Lys1193Val($^{mu/+}$) develop also sarcomas and lymphomas (138). In the tumor cells of mice with *Met* mutations, amplifications of the mutated allele are observed, regardless of the type of mutation (138,139). When knock-in activating alleles are express in the FVB background, tumor latency significantly decrease and a higher penetrance is observed (140). Breast adenocarcinomas are common in all FVB lines, whereas are not observed in C57BL/6 mice. Mice bearing $MET^{Asp1226Asn}$ mutation frequently develop hemangiosarcomas, whereas those with $MET^{Met1248Thr}$ rarely develop sarcomas (140).

The mouse mammary tumor virus (MMTV) promoter allows the expression of MET in the mammary epithelium. The overexpression of MET and its oncogenic isoforms (Met1248Thr, Tyr1003Phe/Met1248Thr) induces tumor formation with low penetrance and a long latency. Mice develop breast carcinomas: 50% with solid nodular histology and 50% with papillary, shirrous, adeno-squamous or spindle cell phenotype (141).

The SWR/J strain of mice is predisposed to develop lung adenomas, spontaneously. This phenomena is under polygenic control and susceptibility alleles have been identified including pulmonary adenoma-susceptibility 1 (Pas1), -resistance 2 and 4 (Par2 and Par4) loci (142). The difference between SWR/J and BALBc mice, respectively susceptible and resistant to develop lung adenomas, depends on the Par4 allele, a narrow region of the murine chromosome 6. Sequencing of this region revealed a single amino-acid change, consisting in a non-conservative Arg968Cys variation in the juxtamembrane domain of met. BALB/c mice carry the Arginine allele, whereas the SWR/J mice strain the Cys variation (142). The corresponding Arg988Cys mutation in humans is observed as germline in one lung adenocarcinoma out of 126 (142) and in 2 SCLC cell lines (92). Arg988Cys mutation increases tumorigenicity in *in vitro* models (92).

Transgenic mice have been created in order to express human HGF exclusively in small airway and therefore the rat Clara cell secretory protein promoter was used (143). These mice develop small airways alterations with congestion, wide bifurcations and increased blood vessels formation. HGF transgenic mice are more susceptible to nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco carcinogen that induces lung tumors. Mice treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, present an increased number of tumors if overexpressing HGF (143). Mice develop papillary adenomas by 10-20 weeks and papillary or solid adenocarcinomas by 30-38 weeks. Tumors arose in this animal model have characteristics of both Clara cells and Type II pneumocytes.

Conclusions

HGF-MET signaling is necessary during embryogenesis for myoblast migration and liver and placenta formation. In adult life, HGF-MET signaling guides tissue repair and exerts protective effects during injury of multiple organs. In tumors, HGF-MET signaling leads to invasion, angiogenesis, metastatic spread, proliferation and anti-apoptosis. The paracrine activation of HGF-MET signal forms a loop, sufficient to sustain tumorigenesis in experimental models. However, genomic aberrations of MET have been described in human tumors; for example amplification and mutations have been reported in in lung adenocarcinomas. Experimental evidences demonstrate that the overexpression of MET and some of its mutants enhance cancer growth. Matter of debate remains which target will be useful for a specific therapy: just the autocrine loop, the protein overexpression, gene amplification or the presence of mutations.

This is relevant for the design of effective clinical trials with anti-MET agents.

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