MET signalling: principles and functions in development, organ regeneration and cancer.

(Article begins on next page)

28 April 2024

UNIVERSITÀ DEGLI STUDI DI TORINO

MET signalling: principles and functions in development, organ regeneration and cancer

Livio Trusolino[1], Andrea Bertotti[1] & Paolo M. Comoglio[1]

[1]Institute for Cancer Research and Treatment (IRCC), University of Torino Medical School, 10060 Candiolo, Torino, Italy.

Correspondence to: Livio Trusolino[1] Email: livio.trusolino@ircc.it
Correspondence to: Paolo M. Comoglio[1] Email: pcomoglio@gmail.com

ABSTRACT

The MET tyrosine kinase receptor (also known as the HGF receptor) promotes tissue remodelling, which underlies developmental morphogenesis, wound repair, organ homeostasis and cancer metastasis, by integrating growth, survival and migration cues in response to environmental stimuli or cell-autonomous perturbations. The versatility of MET-mediated biological responses is sustained by qualitative and quantitative signal modulation. Qualitative mechanisms include the engagement of dedicated signal transducers and the subcellular compartmentalization of MET signalling pathways, whereas quantitative regulation involves MET partnering with adaptor amplifiers or being degraded through the shedding of its extracellular domain or through intracellular ubiquitylation. Controlled activation of MET signalling can be exploited in regenerative medicine, whereas MET inhibition might slow down tumour progression.

Throughout embryogenesis, cells bud off from developing tissues and move outwards to shape and pattern the complex architecture of prospective organs[1]. A similar process occurs in adult life during wound healing and tissue repair, when lingering cells migrate into injury sites to recreate the pre-existing structures[2]. The acquisition of cell motility is necessary, but not sufficient, for this event. Cells that detach from their neighbours must elude anoikis, a form of apoptotic cell death that occurs when cells lose adhesion with the extracellular matrix[3]. Moreover, migratory cells undergo extensive mitotic divisions to produce 'founder populations', which settle in newly forming organs during development or colonize worn tissues during repair[4, 5].

The normal phases of embryogenesis and organ regeneration strongly resemble the pathological process of tumour invasiveness: similarly to cells at the wound edge, cells at the tumour's leading front disrupt intercellular contacts and infiltrate the adjacent surroundings, where they resist anoikis and grow before lodging in the blood vessels for systemic dissemination[6]. This resemblance is not simply a biological correlate, it has a common mechanistic basis: cancer cells resurrect the latent schemes of cellular reorganization, which are usually confined to embryonic development and damaged adult organs, and leverage them to become competent for metastasization[7].

The activities — motility, survival and proliferation — that occur in developing, injured and neoplastic tissues embody a biological programme that is defined as 'invasive growth'[8]. This is triggered by extracellular stimuli that regulate the activity of several transcription factors that, in turn, modulate the expression of a number of proteins, ranging from cytoskeletal and cell–cell junctional components to cell cycle regulators and anti-apoptotic effectors[9, 10]. One major environmental inducer of invasive growth is hepatocyte growth factor (HGF, also known as scatter factor), the ligand for the MET tyrosine kinase receptor (also known as the HGF receptor)[11, 12, 13, 14, 15, 16, 17, 18, 19, 20] (Box 1). MET function is required for various morphogenetic

events in both embryonic and adult life[21, 22] and it drives the malignant progression of several different types of tumours[23]. To do so, MET propagates an intricate system of signalling cascades that result in a comprehensive rewiring of gene expression[24, 25].

The signal transduction biochemistry of MET includes many idiosyncratic details and only a handful of principles that are common to the other tyrosine kinase receptors. Exhaustive information on the identities and branches of MET-dependent signalling networks can be found in numerous reviews[22, 23, 24, 25, 26, 27, 28, 29]. Here, as well as presenting some basic principles of MET signalling regulation, we consider recent findings that have provided fresh knowledge on this matter at the molecular, cellular and animal levels. Specifically, we discuss how the MET pathway is tuned by the functional cooperation between various signal transducers, as well as by the receptor's subcellular localization and trafficking. We also elaborate on how MET signalling influences different organismal functions in normal physiology and disease, a topic that deserves an overall reconceptualization. Finally, we examine the causative relationships between individual signalling inputs and specific biological outputs in MET-driven processes, and provide our perspective on the medical implications of novel therapies that either promote or neutralize MET activity.

MET signalling: pathway components

HGF is secreted as a single-chain inert precursor and converted into a two-chain functional heterodimer by extracellular proteases (Box 1). This growth factor is widely distributed in the extracellular matrix of most tissues, where it is sequestered, mainly in its inactive form, by heparin-like proteoglycans[30, 31]. Cells of mesenchymal origin are the major source of HGF, which acts in a paracrine manner on epithelial cells that express the MET receptor[32]. During tissue repair and cancer invasion, several cytokines that are abundant in the reactive interstitial compartment — for example, interleukin-1 and -6, tumour necrosis factor-α and transforming growth factor-β (TGFβ) — induce transcriptional upregulation of both HGF (in fibroblasts and resident macrophages) and MET (in epithelial cells)[33, 34]. The inflammatory and tumour stroma also overexpress proteases that are involved in pro-HGF activation, such as the plasminogen activation system and matriptase[35, 36]. Thus, biologically competent HGF is not only overproduced but also fully activated. This combined transcriptional and post-translational regulation, which leads to optimal MET activation on target cells, can be considered as part of a general mechanism of physiological defence to tissue damage.

Following HGF binding, the kinase activity of MET is switched on by receptor dimerization and *trans*-phosphorylation of two 'catalytic' tyrosine residues (Tyr1234 and Tyr1235) within the kinase activation loop. The subsequent step is phosphorylation of two additional 'docking' tyrosines in the carboxy-terminal tail (Tyr1349 and Tyr1356), and when phosphorylated, these tyrosines act as a degenerate motif for the recruitment of many signal-relay molecules[37] (Box 1). MET is also a substrate for several protein-tyrosine phosphatases (PTPs), including the receptor PTPs density-enhanced phosphatase 1 (DEP1; also known as PTPRJ) and leukocyte common antigen related (LAR; also known as PTPRF)[38, 39] and the non-receptor PTPs PTP1B (also known as PTPN1) and T cell PTP (TCPTP; also known as PTPN2)[40]. Such phosphatases oppose MET signals by triggering dephosphorylation of either the catalytic tyrosines (in the case of PTP1B and TCPTP)[40] or the docking tyrosines (in the case of DEP1)[38]. To date, the MET tyrosine residues that are specifically dephosphorylated by LAR have not been identified.

This is the basic signalling machinery of MET. Further levels of complexity are provided by the interaction of MET with different signal modifiers, including scaffolding adaptors, cytoskeletal connectors and structurally homologous co-receptors[25, 26, 41] (Fig. 1). As a whole, this apparatus leads to efficient activation of downstream signal transduction pathways that include the mitogen-activated protein kinase (MAPK) cascades (extracellular signal-regulated kinase 1 (ERK1) and

ERK2, Jun amino-terminal kinases (JNKs) and p38), the phosphoinositide 3-kinase–Akt (PI3K–Akt) axis, signal transducer and activator of transcription proteins (STATs), and the nuclear factor-κB inhibitor-α (IκBα)–nuclear factor-κB (NF-κB) complex[22, 25, 42, 43, 44]. All of these pathways positively control MET-dependent cell proliferation, survival and migration. However, in defined cellular contexts, some of these signals can mediate a paradoxical pro-apoptotic, rather than anti-apoptotic, activity (Box 2).

MET and scaffolding adaptors. The promiscuous docking motif in the C-terminal tail of MET binds numerous Src-homology-2 domain (SH2 domain)-containing effectors, such as PI3K[37], the non-receptor tyrosine kinase Src[37], the growth factor receptor-bound protein 2 (GRB2) and SH2 domain-containing transforming protein (SHC) adaptors[37, 45, 46], SHP2 (also known as PTPN11; an upstream activator of Src and Ras)[46], phospholipase Cγ1 (PLCγ1)[37] and the transcription factor STAT3 (Refs 47,48). MET also associates with GRB2-associated-binding protein 1 (GAB1), a multi-adaptor protein that, upon phosphorylation by the MET receptor, provides extra binding sites for SHC, PI3K, SHP2, CRK, PLCγ1 and p120 Ras-GTPase-activating protein (p120-Ras-GAP)[43, 44, 49, 50, 51, 52, 53]. The association between MET and GAB1 occurs directly, through a unique 13-amino-acid MET binding site (MBS) on GAB1, and indirectly, through MET-bound GRB2 (Refs 51,54). Hence, many transducers participate in MET signalling through multiple interactions with the receptor, with the GAB1 scaffolding adaptor or with both (Fig. 1a).

In tumours, intensification of MET signals is also favoured by receptor interaction with the α6β4 integrin. MET activation leads to phosphorylation of the β4-subunit cytoplasmic domain (at three tyrosine residues that are embedded in consensus sequences) and this is required for the recruitment of SHC, PI3K and SHP2 (Refs 55,56,57,58). By accomplishing this function, GAB1 and the α6β4 integrin behave as supplementary docking platforms for the localized recruitment of additional transducers that synergize with those directly bound to MET (Fig. 1a). This mechanism allows signal amplification at the MET-proximal level, which in turn secures potent and enduring activation of downstream transducers.

CD44 links MET to the cytoskeleton. Another protein that cooperates with MET in normal and transformed epithelial cells is CD44, a transmembrane cell adhesion molecule that functions as a linker between the extracellular matrix and the intracellular actin cytoskeleton[59]. Members of the CD44 family differ in their extracellular domain by the insertion of variable regions through alternative splicing, which gives rise to numerous variant isoforms (CD44v1–CD44v10)[59]. The CD44 isoform containing the v6 sequence participates in MET signalling by a twofold mechanism: the extracellular domain is required for the organization of a ternary complex between MET, HGF and CD44 and for the subsequent activation of MET[60]. The cytoplasmic tail is necessary for signal transfer from active MET to Ras[61]. Specifically, upon MET activation, the intracellular domain of CD44v6 drives the assembly of a sub-membraneous network that includes the cytoplasmic region of MET, GRB2, F-actin, ezrin, radixin and moesin (ERM) proteins (which connect actin microfilaments to CD44) and the guanine nucleotide exchange factor son of sevenless (SOS). This network allows for efficient activation of Ras by SOS (Fig. 1b).

In endothelial cells, the v10-containing isoform of CD44 seems to be essential for the partitioning of MET into detergent-insoluble, caveolin-enriched microdomains together with T-lymphoma invasion and metastasis-inducing protein 1 (TIAM1; a RAC1 exchange factor), cortactin (an actin cytoskeletal regulator) and dynamin 2 (a vesicular regulator)[62]. Thus, when MET collaborates with CD44, signal transduction is assisted by its structural and topographical regulation at the inner side of the plasma membrane rather than by its functional interaction with signalling amplifiers. The reasons why only some CD44 variants contribute to MET signalling remain unclear.

Semaphorins activate MET in an HGF-independent manner. The extracellular domain of MET exhibits structural homology with the semaphorins and plexins, a broad family of ligand–receptor pairs that are endowed with either attractive or repulsive function in different tissues (Box 1). In particular, MET and class B plexins share a highly homologous Sema domain, which forms a seven-bladed β-propeller that supports multiple interactions between MET and some class B plexins. When oligomerized with plexins, MET can be activated by semaphorins in an HGF-independent manner, leading to stimulation of MET downstream effectors and execution of MET-dependent biological responses[63, 64, 65] (Fig. 1c). This partnership expands the complement of soluble factors that exert biological activity on MET-expressing cells and specifies the quality of cellular outcomes: cells that co-express MET and plexins react to either HGF or semaphorins by acquisition of invasive ability, which is a typical MET-dependent effect; by contrast, cells that express only plexins respond to semaphorins by arresting migration and inducing cellular collapse, which is a plexin-specific repulsive function[66].

Major MET-regulated signalling pathways

MET-dependent signals are organized in pathways that transmit biochemical information from the cell membrane (where MET resides) to the nucleus (where modulation of gene expression occurs). As described above, the receptor-associated signalling apparatus, including scaffolding adaptors and surface signal modifiers, is somehow unique to MET. Conversely, the downstream transducers are stereotypical signalling modules that are shared among different tyrosine kinase receptors. The prominent examples of these biochemical pathways will now be discussed.

The MAPK cascades. The subfamilies of MAPKs feature a characteristic phospho-relay system in which a series of three protein kinases (MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK) phosphorylate and activate one another[67]. The ERKs are mainly triggered by tyrosine kinase-dependent stimulation of Ras. MET activates Ras through the GRB2–SOS complex, which can interact directly with the multifunctional docking site in the C-terminal tail of MET or can be associated indirectly through the SHC adaptor protein[37,45]. Another route leading to Ras–ERK activation involves the tyrosine phosphatase SHP2, which dephosphorylates the p120-Ras-GAP binding site on GAB1; this impedes the recruitment of p120-Ras-GAP, which usually deactivates Ras, to promote Ras activation[44,53] (Fig. 2a). In the active, GTP-bound state, Ras attracts the Ser/Thr kinase Raf through its effector loop. This association modifies the three-dimensional configuration of Raf, which acquires signalling activity and proceeds to phosphorylate and thereby activate MAPK/ERK kinase 1 (MEK1; also known as MAPKK1) or MEK2 (also known as MAPKK2), the intermediate kinases of the system. In turn, MEK1 and MEK2 phosphorylate ERK1 and ERK2, the final effectors of the cascade. Active ERKs translocate to the nucleus, where they phosphorylate and stabilize several transcription factors that are involved in the early phases of the G1–S cell cycle transition. MET-dependent activation of the Ras–ERK cascade can be decreased by molecules that are involved in organogenesis and tissue repair, such as the membrane receptor Notch and the intracellular modulator Sprouty[68, 69]. Interestingly, MET is able to transcriptionally upregulate both Sprouty and the Notch ligand Delta, suggesting a mechanism of counter-feedback whereby MET induces negative regulators of its own signalling, possibly to fine-tune the restricted execution of biological outputs in space and time. How the Delta–Notch system and Sprouty impair MET-triggered stimulation of the Ras–ERK pathway remains to be characterized.

As well as the ERKs, MET is able to activate the JNK and p38 MAPKs. For both families, the first MAPKKK components of the cascade (MAPKKK1–MAPKKK4; also known as MEK kinase 1 (MEKK1)–MEKK4) are usually stimulated by Rac, a small GTPase that is switched on by a Ras–PI3K-mediated pathway. MEKK-dependent phosphorylation of MEK4 and MEK7 leads to activation

of JNK1, JNK2 and JNK3, whereas phosphorylation of MEK3 and MEK6 leads to activation of p38α, p38β, p38γ and p38δ (Fig. 2a). Following MET-dependent stimulation, the JNKs and p38s control a range of cellular processes as diverse as cell proliferation, differentiation, transformation and apoptosis[70, 71, 72, 73, 74].

The PI3K–Akt axis. PI3K can be activated directly by MET and/or indirectly by Ras, the activation of which can also be mediated by MET (see above)[37]. PI3K activation results in the formation of phosphatidylinositol-3,4,5-triphosphate, which tethers pleckstrin homology domain (PH domain)-containing molecules, such as the Ser/Thr kinase Akt, to the plasma membrane. Akt is then able to suppress apoptosis through inactivation of the pro-apoptotic protein BCL-2 antagonist of cell death (BAD) and activation of the E3 ubiquitin-protein ligase MDM2, which promotes the degradation of the pro-apoptotic protein p53. Moreover, Akt inactivates glycogen synthase kinase 3β (GSK3β), which antagonizes the expression of positive cell cycle regulators such as Myc and cyclin D1, and activates mammalian target of rapamycin (mTOR), which stimulates protein synthesis and physical cell enlargement (Fig. 2b).

The STAT pathway. *Trans*-phosphorylation of the docking site of MET leads to the association of STATs — namely, STAT3 — with its tail[47, 48]. This docking event is followed by MET-dependent tyrosine phosphorylation of STAT3, which causes STATs to dissociate from the receptors and homodimerize through their SH2 domains. Eventually, STAT dimers translocate to the nucleus, where they operate as transcription factors to regulate the expression of several genes that are implicated in cell proliferation or differentiation (Fig. 2c).

The IκBα–NF-κB complex. The NF-κB system comprises a family of transcription factors that are held inactive in the cytoplasm by a family of inhibitory proteins known as IκBs[75]. These inhibitors are ubiquitylated and degraded by a phosphorylation event that is triggered by the IκB kinase (IKK). In response to MET, IKK activation is mediated by the PI3K–Akt pathway and Src as signalling intermediates, but the direct distal effectors of IKK stimulation are unknown[76]. IKK-induced destruction of IκBs leads to the release of NF-κB, which translocates to the nucleus to stimulate the transcription of various genes, including mitogenic and anti-apoptotic regulators[76, 77] (Fig. 2d).

Regulation of MET signalling

It is well established that signal transduction pathways that are downstream of tyrosine kinase receptors are modulated not only by the identities of protein–protein interactions but also by the colocalization of signal transducers in membrane domains or, more broadly, in organelles and vesicles[78]. This notion has been recently applied to MET: the prevailing view that the activated receptor recruits signalling effectors at the plasma membrane, which in turn stimulate diffusible intermediates in the cytosol, has now been expanded by the finding that MET signals can also emanate from endosomal compartments. Moreover, the levels of MET expression at the cell surface are finely tuned by other spatially restricted events, including extracellular shedding, intracellular cleavage and ubiquitin-mediated degradation. All of these processes regulate the strength of MET activation and the ensuing robustness of MET-dependent signals.

MET internalization can promote signalling. Following ligand binding, MET is rapidly internalized by clathrin-mediated endocytosis and becomes recruited to peripheral early endosomes[79]. Here, MET triggers the sustained activation of ERKs, which are then relocated to focal complexes, where they mediate HGF-induced cell migration (probably by phosphorylating paxillin and other focal adhesion targets[80]). A crucial regulator of this endosomal signal

compartmentalization, and the consequent transfer of ERK to adhesion sites, is protein kinase Cε (PKCε) (Fig. 3a). RNA interference-mediated silencing of PKCε negatively influences the recruitment of ERK1 and ERK2 to focal adhesions[81], possibly by reducing the delivery of ERK–integrin complexes to the plasma membrane[82].

From the peripheral endosomes, MET traffics along the microtubule network and accumulates in a nondegradative perinuclear endomembrane compartment through a process that is promoted by PKCα[81, 83] (Fig. 3b). This juxtanuclear delivery of MET is required for the optimal activation and nuclear translocation of STAT3 (Ref. 84). MET-triggered phosphorylation of STAT3 at the plasma membrane or early endosomes is likely to be below the threshold for nuclear uptake owing to the intense local activity of phosphotyrosine phosphatases that dissipate STAT3 signal intensity during protein diffusion. Conversely, post-endocytic trafficking to the perinuclear compartment brings active MET into close proximity to the nucleus, which shields STAT3 against phosphatase activity and allows its nuclear accumulation (Fig. 3b).

MET internalization can inhibit signalling. Although internalized receptors may remain able to signal in peripheral and perinuclear compartments, the endocytosis of ligand-activated receptors and their subsequent degradation in the lysosomal compartment remains the major determinant of receptor desensitization and signal restraint[85]. Prompt sequestration of the activated receptor into invaginating pits, and its endosomal sorting for degradation, precludes prolonged signal emission and ensures faithful application of physiological responses.

Trafficking to the lysosomes is triggered by ligand-induced activation of MET, and requires the function of casitas B-lineage lymphoma (CBL), an E3 ubiquitin-protein ligase that is recruited to a juxtamembrane phosphotyrosine residue in the active receptor (Tyr1003)[86](Fig. 3c). This leads to MET being monoubiquitylated at multiple sites[87], which enables its recognition by endocytic adaptors that contain ubiquitin-binding domains, its sorting into clathrin-coated areas at the cell surface and its delivery to the endosomal network. From these sorting endosomes, MET accumulates on the limiting and internal membranes of multivesicular bodies. This terminates signalling by sequestering MET from the cytosol and preventing it being recycled by the plasma membrane. Finally, the multivesicular bodies fuse with the lysosomes and MET undergoes proteolytic demolition[88, 89]. Seemingly active MET is not polyubiquitylated, and is therefore unlikely to be targeted for degradation by the proteasome[87]; however, proteasomal destruction of MET can occur in an ubiquitin-independent manner (see below).

CBL also attracts — through the scaffold molecule CBL-interacting protein 85 (CIN85; also known as SH3KBP1) — endophilins, a family of proteins that assist the negative curvature, invagination and scission of the planar plasma membrane during the early phases of endocytosis[90]. Therefore, CBL promotes the physical internalization of MET while tagging it for lysosomal degradation[91] (Fig. 3c). Sustained association of CBL with active MET is favoured by decorin, an extracellular proteoglycan that binds directly to MET with high affinity and acts as an antagonistic ligand by stimulating receptor activation and, immediately thereafter, receptor downmodulation[92].

What skews the MET fate towards degradation rather than signalling remains unknown. In the case of the epidermal growth factor (EGF) receptor, it has been suggested that endosomal signalling predominates under conditions of scarce ligand, whereas the degradative pathway is favoured when abundant ligand is present[93]. A similar scenario might also apply to MET but a formal demonstration of such a mechanism has not been provided so far.

Regulated proteolysis of MET. Downregulation of MET signalling also involves sequential proteolytic cleavages. The first step is a disintegrin and metalloprotease (ADAM)-mediated release (known as shedding) of the extracellular domain, which generates a soluble N-terminal fragment

and a membrane-anchored cytoplasmic tail. Then, the surface-associated cytoplasmic remnant undergoes regulated proteolysis by γ-secretase, which yields a labile intracellular portion that is rapidly cleared by proteasome-mediated degradation[94] (Fig. 3d).

Unlike CBL-mediated endosomal degradation, regulated proteolysis of MET is ligand- and ubiquitin-independent and does not require the kinase activity of the receptor: the mechanism occurs basally and affords MET signalling with chronic, low-grade attenuation under steady-state conditions. The shedding of MET that is catalysed by ADAM metalloproteases can be acutely enhanced by various agents such as phorbol esters, suramin, lysophosphatidic acid and monoclonal antibodies (mAbs) against the MET ectodomain[94, 95, 96, 97, 98, 99, 100, 101, 102]. Importantly, 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 (by sequestering the ligand) and full-length MET (by impairing dimerization and transactivation of the native receptor) to further inhibit MET signalling[103, 104]. Therefore, the use of targeted agents — such as mAbs, which increase MET shedding from the cell surface — could have a great therapeutic effect on cancers by reducing MET signalling.

MET signalling in development and disease

To secure output production and to increase functional versatility in the face of recurring perturbations, tyrosine kinase receptors often expand and diversify individual components of their downstream signalling networks, degenerate connections and duplicate entire modules of protein–protein interactions. Signal redundancy, reciprocal signal reinforcement and feedback loops are stereotypical aspects of signal transduction[105]. Because of this, extracting functional information from the dissection of individual signalling axes might be judged to be simplistic, if not futile. Signal compensation by other transducers that have overlapping functions must be considered. However, in the case of MET, different cell- and tissue-specific activities seem to be fulfilled by dedicated signalling cascades, with some transducers dominating over others depending on context, timing and biological complexity (Table 1). Here, we outline a number of genetic and pharmacologic studies that have contributed to this knowledge. The coverage is purposefully not exhaustive: we will limit our attention to those settings in which a more documented connection between signals and phenotypes has been provided, as well as to those for which information has become available only recently.

Embryonic development. During development, HGF and MET convey essential signals for the growth and survival of hepatocytes and placental trophoblast cells. In *Hgf*- or *Met*-null embryos, the liver is considerably reduced in size and the placental labyrinth is severely hypomorphic[106, 107, 108] (Fig. 4). This hypomorphic phenotype eventually leads to death *in utero* owing to compromised exchange of oxygen and nutrients between maternal and fetal blood. The HGF–MET system also has a decisive role in the proliferation and motility of long-range migrating muscle progenitors. These myogenic precursors delaminate from the dermomyotome, a structure in the dorsolateral region of somites, and travel to the limbs, tongue and diaphragm, where they differentiate to form a subset of the hypaxial muscles[109]. Ablation of the *Hgf* or *Met* genes results in complete absence of this specific muscle type, leaving all other muscle groups unaffected[104, 105, 106]. Finally, disruption of HGF–MET function affects the proper wiring of the nervous system, leading to reduced survival of sensory and sympathetic neurons as well as impaired outgrowth and fasciculation (axon bundling) of certain motor nerves[110, 111, 112].

Using genetic knock-in approaches, several studies have addressed the physiological significance of the different signalling pathways that are regulated by MET in the whole organism. Specificity-switch mutants, in which the MET multifunctional docking site has been converted into an optimal binding motif for PI3K, Src or GRB2, exhibit a rescue of defined phenotypes (when compared with *Met*-null animals) in line with the effectors that are recruited. Mice in which MET exclusively associates with GRB2 are viable, fertile and apparently normal — indicating that GRB2 is sufficient to recruit all of the necessary downstream effectors of MET — whereas mutants with dedicated stimulation of Src or PI3K are embryonic lethal. However, although incompatible with survival, individual activation of Src is sufficient to restore proliferation of placental trophoblasts and myoblasts, and selective activation of PI3K is sufficient to promote axon outgrowth and branching of specific motor nerves[113]. Together, these findings suggest that MET-mediated developmental events are accomplished by qualitatively different signals that are endowed with a tissue-specific activity.

The defects that are observed in *Hgf* and *Met* knockout embryos are phenocopied in *Gab1*-null mice — albeit in a slightly attenuated form — indicating that GAB1 has an essential role in MET-based signal transduction pathways[114] (Fig. 4). As mentioned above, GAB1 associates with MET directly, through a specific MBS, and indirectly, through the GRB2 adaptor[51, 54]. The mechanism of recruitment of GAB1 to MET influences biological outcomes *in vivo* in a tissue-specific manner: both direct and indirect interactions with MET are necessary for functional MET signalling during liver and placenta formation, whereas either the GRB2 binding site or the MBS, but not both, are required for limb muscle development[115]. Similar to those observed for MET, interactions of GAB1 with specific downstream effectors control distinct developmental processes. The association between GAB1 and PI3K seems to be essential for EGF receptor-mediated embryonic eyelid closure but this association is not involved in MET-regulated organogenesis[113]. MET signalling in embryonic development is mainly channelled by the GAB1–SHP2 interaction; indeed, GAB1 mutants that are unable to bind SHP2 phenotypically copy *Met*-null mutants, with defects such as a thin placental labyrinth layer (Fig. 4) and impaired migration of muscle precursors[115]. Again, we can conclude that the biological responses elicited by GAB1, like those elicited by MET, have different signal requirements in different tissues during embryonic development.

Organ regeneration. The signalling mechanisms that mediate MET-driven tissue patterning during organ regeneration have been studied both *in vitro*, by performing morphogenetic assays under three-dimensional culture conditions, and *in vivo*, using Cre–*loxP*-mediated conditional deletion of MET in selected tissues.

When embedded in a collagen matrix, normal epithelial cells proliferate and assemble into spheroids, which feature a centrally localized, hollow lumen, surrounded by polarized cells[116]. HGF causes spheroids to form elongated tubules in a stepwise sequence of biological events[117]. First, cells undergo a partial epithelial–mesenchymal transition (EMT), which involves the transformation of tightly packed elements into invasive, spindle-shaped cells that emit long cytoplasmic extensions in the surrounding matrix. Then, these extensions develop into single-file chains, which must resist anoikis to lengthen and thicken. Ultimately, cells redifferentiate: they restore polarity and turn into solid cords that become mature tubules by the progressive formation of a continuous lumen. At the signalling level, the series of events required for HGF-dependent tubule formation has been dissected. As a whole, this process requires the integrity of the STAT3 and NF-κB pathways[47, 77]. The initial EMT relies on hypersustained activation of the Raf–MEK–ERK pathway, which, as discussed earlier, is triggered by the association of active MET-bound GAB1 with the tyrosine phosphatase SHP2 (Refs 44,53,118,119). Cell survival is promoted by transcription factors of the Snail family[120]. The later phases of epithelial tubulogenesis entail the activity of matrix metalloproteases (MMPs), which, albeit dispensable for the early formation of extensions and

chains, seem to be necessary to produce cords and hollow tubules[118]. HGF has an important role in the regulation of the functions of MMPs by enhancing the transcriptional levels of a large number of MMPs as well as those of several proteases that convert the precursor forms of MMPs into active enzymes[121, 122, 123].

In vivo, MET function is particularly important for the regeneration of the liver and the kidney in response to both acute and chronic insults. Production of HGF increases steeply following liver damage, and the ensuing activation of MET in hepatocytes provides strong mitogenic and anti-apoptotic stimuli for organ repair[14, 15, 124]. Accordingly, conditional deletion of MET in the liver results in impaired organ reconstitution after toxic insult and hepatectomy[125, 126] (Fig. 4). In these mutant mice, phosphorylation of ERK1 and ERK2 is lost and there is a delay in the timing of maximal activation of Akt that occurs after hepatic injury. The proliferation defect is reflected by low transcriptional induction of cyclins and enhanced expression of the cyclin-dependent kinase (CDK) inhibitor p21 (also known as CIP1 and WAF1)[126]. An analogous phenotype of compromised hepatic regeneration is also seen in mice with liver-specific ablation of GAB1 or SHP2 (Ref. 127), further pointing to a crucial role for GAB1–SHP2 signalling in MET-driven outcomes (Fig. 4).

MET also displays a similar protective activity in the kidney. HGF induces intense proliferative and anti-apoptotic responses in renal epithelial cells by activating Ras-dependent mitogenic signals, the PI3K–Akt pro-survival pathway and, subsequently, the transcriptional upregulation of the anti-apoptotic effectors B-cell lymphoma-extra large (BCL-XL) and BCL-2 (Ref. 128). This powerful reno-protective activity prevents acute renal failure due to tubular necrosis and accelerates kidney regeneration.

In the liver and the kidney, persistent damage leads to a severe fibrotic evolution that is sustained by overproduction of TGFβ, which instructs interstitial myofibroblasts to deposit copious amounts of extracellular matrix[129]. HGF acts as a powerful anti-fibrotic agent by antagonizing TGFβ fibrogenic responses in different ways, from transcriptional downmodulation of TFGβ[130] to ERK-mediated inhibition of SMADs[131, 132], the cardinal transcriptional effectors of TGFβ signalling. At least in the liver, fibrosis is further reduced by an atypical pro-apoptotic effect of HGF on myofibroblasts[133] (Box 2).

Epithelial cells also exploit the trophic properties of the HGF–MET system during wound healing. At the wound edges, marginal keratinocytes form the so-called hyperproliferative epithelium, which undergoes rounds of cell division to provide fresh elements that migrate over the provisional matrix of the injured dermis and repopulate the wounded area[134]. Keratinocytes from mutant mice with conditional deletion of MET in the epidermis are unable to contribute to the generation of the hyperproliferative epithelium *in vivo*[135]. *In vitro*, during the closure of scratch wounds, HGF is necessary to re-orient keratinocytes, so that focal adhesion components, actin stress fibres and the plus ends of microtubules point towards the wound edges as a prelude for productive cell locomotion. Moreover, HGF fosters the proliferation and migration of epithelial cells into the de-epithelialized zone. The major signalling effectors that mediate these processes are GAB1, Akt, ERK1 and ERK2, as well as p21-activated kinase 1 (PAK1) and PAK2, targets of RhoA that control actin polymerization and protrusion formation[135].

Cancer growth and metastasis. In most tumours, MET is transcriptionally induced by hypoxia and inflammatory cytokines or pro-angiogenic factors that are abundant in the reactive stroma of full-blown tumours[34, 136]. Hence, MET activation is a late event that aggravates the intrinsic malignant properties of already transformed cells by conveying proliferative, anti-apoptotic and pro-migratory signals (a biological situation that our group calls 'oncogene expedience')[23].

Congruent with the view that invasive cancer cells resume behavioural programmes that are normally operative during embryogenesis and in damaged tissues, MET-driven exacerbation of cancer progression involves stimulation of all the signalling pathways that are known to mediate

the several aspects of MET physiological responses in development and adult life. The contribution of specific signals to MET activity in tumours has been partially dissected through the manipulation of the consensus sequences that mediate the recruitment of distinct transducers. By converting the multifunctional docking site of an oncogenic mutant of MET into preferential binding motifs for GRB2 or PI3K, it was demonstrated that Ras signals are primarily involved in MET-triggered cell proliferation, whereas PI3K recruitment is required for the induction of cell motility and invasion; however, a fully metastatic phenotype can be recapitulated only when both effectors are concomitantly associated with MET[137, 138]. Thus, combined activation of multiple pathways is necessary to instruct the full execution of MET-dependent invasive growth in cancer cells. However, blocking a single network node is sufficient to perturb cellular responses, indicating that the targeted inhibition of a specific signalling pathway cannot be compensated for by other, still active, regulatory tiers. This is in line with the well-established observation that obstruction of individual MET-dependent pathways adversely affects tumour growth, survival and migration in various cancer cell types and under different experimental settings[37, 48, 50, 56, 57, 58,70, 71, 72, 76, 139]. For example, the integrity of JNK- and p38-dependent signals is required for MET-stimulated proliferation and anchorage-independent growth in MET-transformed fibroblasts and melanoma cells[70, 71, 72], and STAT3 signalling and NF-κB activity are necessary for MET-induced onset of leiomyosarcomas and for the survival of prostate cancer cells, respectively[48, 76].

The notion that inhibiting a single transducer will suffice to revert or impair the biological phenotype induced by MET activation has strong implications. If there is no redundancy in the many signalling networks regulated by MET, this implicates that targeting any of these pathways would hamper one or more aspects of MET-driven cancer progression, which could pave the way for the development of several therapeutic strategies together with, or as an alternative to, the selective inhibition of MET. However, recent data suggest that this is unlikely to be the case. In a limited number of tumour cells featuring genetic lesions of MET — caused by gene amplification and, possibly, point mutations[23, 140, 141] — receptor hyperactivation is inherent in the cancer's natural history and is required to maintain the transformed phenotype: these cells are dependent on the persistent activity of MET for their relentless proliferation (a situation known as 'oncogene addiction')[142, 143]. In this scenario, MET blockade affects a limited subset of MET downstream signals: many of the pathways controlling MET-driven responses — including STATs, JNK, p38 and NF-κB — remain active or exhibit scant responses, and only a restricted complement of Ras and PI3K transducers and transcriptional effectors is neutralized[144, 145, 146]. Of note, a similar response to oncogene inactivation also occurs in cells that depend on the EGF receptor for their growth and survival, suggesting that cells that are 'addicted' to oncogenic tyrosine kinase receptors may develop common mechanisms to sustain malignancy and therefore may be susceptible to similar therapeutic interventions[144, 145].

The mechanisms that are responsible for this kind of selectivity have not been elucidated, but they could rely on feedback effects that are hard-wired into oncogene-addicted tumours. In the case of the EGF receptor, transcriptional induction of the dual specificity phosphatases (DUSPs), which are negative regulators of MAPK activity, has been proposed as a crucial step in modulating receptor signalling outputs[147]. Interestingly, DUSPs exert a promiscuous phosphatase activity on multiple members of the MAPK family[148]. One could speculate that an imbalance in substrate dephosphorylation might lead to the silencing of some MAPK components while leaving others relatively unaffected.

Intrinsic addiction to MET is characterized by high steady-state signalling due to chronic receptor activation, which is the consequence of a fixed and transmissible genetic alteration; in this setting, the reliance of tumours on the continued activity of MET seems to be governed by a small, self-sufficient group of signal transducers. Conversely, the activation state of MET in the context of oncogene expedience is dynamic because it mainly depends on the hectic and reversible

environmental conditions that are typical of cancer; in this situation, MET-regulated outcomes are controlled by multiple, non-redundant signalling crosstalks. This implies that oncogene addiction and oncogene expedience should be considered as separate entities when managing therapeutic opportunities and future strategies against cancer, and in particular when designing prospective combination therapies.

Outlook

Embryonic morphogenesis, organ regeneration and cancer invasion are all facets of a common theme in which MET and MET signals have fundamental roles. Although the regulatory molecules are the same and the biological outcomes share unifying principles, the precise mechanisms that dictate the final execution of one or another response are far from being elucidated. The regulation of the different outcomes of MET activation, as well as the definition of their biological specificity, could depend on signal modifiers that are endowed with a tissue-restricted distribution (including, as recently demonstrated, microRNAs[149,150, 151, 152, 153]) or on dedicated protein interaction networks, and could be further tuned by transcriptional modulation.

The ongoing development of new technologies is certainly one prerequisite for unravelling the complexity of these issues. Cell- and context-dependent signal modulators can be now identified by using genome-wide functional screens; higher details in regulatory protein–protein interactions can be achieved by using mass spectrometry; and, finally, information on the presence of positive or negative signalling feedback loops, which are likely to enforce or attenuate MET biochemical outputs, can be extracted from gene expression profiling. The long catalogues of hits pinpointed by such 'omics' efforts can be prioritized by using bioinformatics and challenged by using high-throughput platforms for biological validation.

Gathering this kind of knowledge has important medical relevance. Due to the ability of MET to support organ repair and contrast fibrotic evolution, agents that are endowed with agonistic activity are currently being tested in a number of early-phase clinical trials. From an opposite perspective, several MET inhibitors are being evaluated for their efficacy in blocking the growth and progression of various tumour types[23]. Most anti-cancer therapies are accompanied by drug-induced toxicity that could be relieved by triggering MET regenerative signals in the damaged organs; ironically, this approach is hindered by the reasonable concern that systemic delivery of MET-activating compounds in cancer patients would promote the spread of neoplastic cells and awaken dormant metastases[154]. Even more insidiously, MET-driven stimulation of growth and survival signals might cause the progression of incipient pre-malignant clones to full-blown malignancy in apparently healthy individuals. Detailed knowledge of MET-regulated signals *in vivo* will prove useful to identify the pathways that preferentially mediate the healing properties of MET and those that convey its lethal pro-invasive activity. If successful, this endeavour will provide momentum for clinical applications in both regenerative medicine and cancer therapy.

Competing interests statement

The authors declare no competing financial interests.

References

1. Bryant, D. M. & Mostov, K. E. From cells to organs: building polarized tissue. *Nature Rev. Mol. Cell Biol.* 9, 887–901 (2008).

2. Gurtner, G. C. , Werner, S. , Barrandon, Y. & Longaker, M. T. Wound repair and regeneration. *Nature* 453, 314–321 (2008).

3. Frisch, S. M. & Screaton, R. A. Anoikis mechanisms. *Curr. Opin. Cell Biol.* 13, 555–562 (2001).

4. Lu, P. & Werb, Z. Patterning mechanisms of branched organs. *Science* 322, 1506–1509 (2008).

5. Zaret, K. S. & Grompe, M. Generation and regeneration of cells of the liver and pancreas. *Science* 322, 1490–1494 (2008).

6. Schäfer, M. & Werner, S. Cancer as an overhealing wound: an old hypothesis revisited.*Nature Rev. Mol. Cell Biol.* 9, 628–638 (2008).

7. Yang, J. & Weinberg, R. A. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev. Cell* 14, 818–829 (2008).

8. Christofori, G. New signals from the invasive front. *Nature* 441, 444–450 (2006).

9. Kalluri, R. & Weinberg, R. A. The basics of epithelial-mesenchymal transition. *J. Clin. Invest.* 119, 1420–1428 (2009).

10. Thiery, J. P. , Acloque, H. , Huang, R. Y. & Nieto, M. A. Epithelial-mesenchymal transitions in development and disease. *Cell* 139, 871–890 (2009).

11. Park, M. *et al.* Mechanism of *met* oncogene activation. *Cell* 45, 895–904 (1986).

12. Giordano, S. , Ponzetto, C. , Di Renzo, M. F. , Cooper, C. S. & Comoglio, P. M. Tyrosine kinase receptor indistinguishable from the c-*met* protein. *Nature* 339, 155–156 (1989).

13. Stoker, M. , Gherardi, E. , Perryman, M. & Gray, J. Scatter factor is a fibroblast-derived modulator of epithelial cell motility. *Nature* 327, 239–242 (1987).

14. Nakamura, T. *et al.* Molecular cloning and expression of human hepatocyte growth factor. *Nature* 342, 440–443 (1989).

15. Zarnegar, R. & Michalopoulos, G. Purification and biological characterization of human hepatopoietin A, a polypeptide growth factor for hepatocytes. *Cancer Res.* 49, 3314–3320 (1989).

16. Bottaro, D. P. *et al.* Identification of the hepatocyte growth factor receptor as the c-*met* proto-oncogene product. *Science* 251, 802–804 (1991).

17. Montesano, R. , Matsumoto, K. , Nakamura, T. & Orci, L. Identification of a fibroblast-derived epithelial morphogen as hepatocyte growth factor. *Cell* 67, 901–908 (1991).

18. Naldini, L. *et al.* Hepatocyte growth factor (HGF) stimulates the tyrosine kinase activity of the receptor encoded by the proto-oncogene c-MET. *Oncogene* 6, 501–504 (1991).

19. Naldini, L. *et al.* Scatter factor and hepatocyte growth factor are indistinguishable ligands for the MET receptor. *EMBO J.* 10, 2867–2878 (1991).

20. Weidner, K. M. *et al.* Evidence for the identity of human scatter factor and human hepatocyte growth factor. *Proc. Natl Acad. Sci. USA* 88, 7001–7005 (1991).

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

22. Birchmeier, C. , Birchmeier, W. , Gherardi, E. & Vande Woude, G. F. Met, metastasis, motility and more. *Nature Rev. Mol. Cell Biol.* 4, 915–925 (2003).

23. Comoglio, P. M. , Giordano, S. & Trusolino, L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. *Nature Rev. Drug Discov.* 7, 504–516 (2008).

24. Trusolino, L. & Comoglio, P. M. Scatter-factor and semaphorin receptors: cell signalling for invasive growth. *Nature Rev. Cancer* 2, 289–300 (2002).

25. Lai, A. Z. , Abella, J. V. & Park, M. Crosstalk in Met receptor oncogenesis. *Trends Cell Biol.* 19, 542–551 (2009).

26. Furge, K. A. , Zhang, Y. W. & Vande Woude, G. F. Met receptor tyrosine kinase: enhanced signaling through adapter proteins. *Oncogene* 19, 5582–5589 (2000).

27. Zhang, Y. W. & Vande Woude, G. F. HGF/SF-met signaling in the control of branching morphogenesis and invasion. *J. Cell. Biochem.* 88, 408–417 (2003).

28. Gao, C. F. & Vande Woude, G. F. HGF/SF-Met signaling in tumor progression. *Cell Res.*15, 49–51 (2005).

29. Peschard, P. & Park, M. From Tpr–Met to Met, tumorigenesis and tubes. *Oncogene* 26, 1276–1285 (2007).

30. Kobayashi, T. *et al.* Hepatocyte growth factor specifically binds to sulfoglycolipids. *J. Biol. Chem.* 269, 9817–9821 (1994).

31. Lyon, M. , Deakin, J. A. , Mizuno, K. , Nakamura, T. & Gallagher, J. T. Interaction of hepatocyte growth factor with heparan-sulfate. Elucidation of the major heparan sulfate structural determinants. *J. Biol. Chem.* 269, 11216–11223 (1994).

32. Sonnenberg, E. , Meyer, D. , Weidner, K. M. & Birchmeier, C. Scatter factor/hepatocyte growth factor and its receptor, the c-met tyrosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development. *J. Cell Biol.*123, 223–235 (1993).

33. Michalopoulos, G. K. & DeFrances, M. C. Liver regeneration. *Science* 276, 60–66 (1997).

34. Bhowmick, N. A. , Neilson, E. G. & Moses, H. L. Stromal fibroblasts in cancer initiation and progression. *Nature* 432, 332–337 (2004).

35. Mars, W. M. , Zarnegar, R. & Michalopoulos, G. K. Activation of hepatocyte growth factor by the plasminogen activators uPA and tPA. *Am. J. Pathol.* 143, 949–958 (1993).

36. Owen, K. A. *et al.* Pericellular activation of hepatocyte growth factor by the transmembrane serine proteases matriptase and hepsin, but not by the membrane-associated protease uPA. *Biochem. J.* 426, 219–228 (2010).

37. Ponzetto, C. *et al.* A multifunctional docking site mediates signaling and transformation by the hepatocyte growth factor/scatter factor receptor family. *Cell* 77, 261–271 (1994).

38. Palka, H. L. , Park, M. & Tonks, N. K. Hepatocyte growth factor receptor tyrosine kinase met is a substrate of the receptor protein-tyrosine phosphatase DEP-1. *J. Biol. Chem.* 278, 5728–5735 (2003).

39. Machide, M. , Hashigasako, A. , Matsumoto, K. & Nakamura, T. Contact inhibition of hepatocyte growth regulated by functional association of the c-Met/hepatocyte growth factor receptor and LAR protein-tyrosine phosphatase. *J. Biol. Chem.* 281, 8765–8772 (2006).

40. Sangwan, V. *et al.* Regulation of the Met receptor-tyrosine kinase by the protein-tyrosine phosphatase 1B and T-cell phosphatase. *J. Biol. Chem.* 283, 34374–34383 (2008).

41. Bertotti, A. & Comoglio, P. M. Tyrosine kinase signal specificity: lessons from the HGF receptor. *Trends Biochem. Sci.* 28, 527–533 (2003).

42. Sipeki, S. *et al.* Phosphatidylinositol 3-kinase contributes to Erk1/Erk2 MAP kinase activation associated with hepatocyte growth factor-induced cell scattering. *Cell Signal.*11, 885–890 (1999).

43. Maroun, C. R. *et al.* The Gab1 PH domain is required for localization of Gab1 at sites of cell-cell contact and epithelial morphogenesis downstream from the met receptor tyrosine kinase. *Mol. Cell. Biol.* 19, 1784–1799 (1999).

44. Maroun, C. R. , Naujokas, M. A. , Holgado-Madruga, M. , Wong, A. J. & Park, M. The tyrosine phosphatase SHP-2 is required for sustained activation of extracellular signal-regulated kinase and epithelial morphogenesis downstream from the met receptor tyrosine kinase. *Mol. Cell. Biol.* 20, 8513–8525 (2000).

45. Pelicci, G. *et al.* The motogenic and mitogenic responses to HGF are amplified by the Shc adaptor protein. *Oncogene* 10, 1631–1638 (1995).

46. Fixman, E. D. , Fournier, T. M. , Kamikura, D. M. , Naujokas, M. A. & Park M. Pathways downstream of Shc and Grb2 are required for cell transformation by the Tpr–Met oncoprotein *J. Biol. Chem.* 271, 13116–13122 (1996)

47. Boccaccio, C. *et al.* Induction of epithelial tubules by growth factor HGF depends on the STAT pathway. *Nature* 391, 285–288 (1998).

48. Zhang, Y. W. , Wang, L. M. , Jove, R. & Vande Woude, G. F. Requirement of Stat3 signaling for HGF/SF-Met mediated tumorigenesis. *Oncogene* 21, 217–226 (2002).

49. Weidner, K. M. *et al.* Interaction between Gab1 and the c-Met receptor tyrosine kinase is responsible for epithelial morphogenesis. *Nature* 384, 173–176 (1996).

50. Garcia-Guzman, M. , Dolfi, F. , Zeh, K. & Vuori, K. Met-induced JNK activation is mediated by the adapter protein Crk and correlates with the Gab1–Crk signaling complex formation. *Oncogene* 18, 7775–7786 (1999).

51. Schaeper, U. *et al.* Coupling of Gab1 to c-Met, Grb2, and Shp2 mediates biological responses. *J. Cell Biol.* 149, 1419–1432 (2000).

52. Gual, P. *et al.* Sustained recruitment of phospholipase c-γ to Gab1 is required for HGF-induced branching tubulogenesis. *Oncogene* 19, 1509–1518 (2000).

53. Montagner, A. *et al.* A novel role for Gab1 and SHP2 in epidermal growth factor-induced Ras activation. *J. Biol. Chem.* 280, 5350–5360 (2005).

54. Lock, L. S. , Royal, I. , Naujokas, M. A. & Park, M. Identification of an atypical Grb2 carboxyl-terminal SH3 domain binding site in Gab docking proteins reveals Grb2-dependent and -independent recruitment of Gab1 to receptor tyrosine kinases. *J. Biol. Chem.* 275, 31536–31545 (2000).

55. Comoglio, P. M. , Boccaccio, C. & Trusolino, L. Interactions between growth factor receptors and adhesion molecules: breaking the rules. *Curr. Opin. Cell Biol.* 15, 565–571 (2003).

56. Trusolino, L. , Bertotti, A. & Comoglio, P. M. A signaling adapter function for α6β4 integrin in the control of HGF-dependent invasive growth. *Cell* 107, 643–654 (2001).

57. Bertotti, A. , Comoglio, P. M. & Trusolino, L. β4 integrin is a transforming molecule that unleashes Met tyrosine kinase tumorigenesis. *Cancer Res.* 65, 10674–10679 (2005).

58. Bertotti, A. , Comoglio, P. M. & Trusolino, L. β4 integrin activates a Shp2-Src signaling pathway that sustains HGF-induced anchorage-independent growth. *J. Cell Biol.* 175, 993–1003 (2006).

59. Ponta, H. , Sherman, L. & Herrlich, P. A. CD44: from adhesion molecules to signalling regulators. *Nature Rev. Mol. Cell Biol.* 4, 33–45 (2003).

60. Orian-Rousseau, V. , Chen, L. , Sleeman, J. P. , Herrlich, P. & Ponta, H. CD44 is required for two consecutive steps in HGF/c-Met signaling. *Genes Dev.* 16, 3074–3086 (2002).

61. Orian-Rousseau, V. *et al.* Hepatocyte growth factor-induced Ras activation requires ERM proteins linked to both CD44v6 and F-actin. *Mol. Biol. Cell* 18, 76–83 (2007).

62. Singleton, P. A. *et al.* CD44 regulates hepatocyte growth factor-mediated vascular integrity. Role of c-Met, Tiam1/Rac1, dynamin 2, and cortactin. *J. Biol. Chem.* 282, 30643–30657 (2007).

63. Giordano, S. *et al.* The semaphorin 4D receptor controls invasive growth by coupling with Met. *Nature Cell Biol.* 4, 720–724 (2002).

64. Conrotto, P. , Corso, S. , Gamberini, S. , Comoglio, P. M. & Giordano, S. Interplay between scatter factor receptors and B plexins controls invasive growth. *Oncogene* 23, 5131–5137 (2004).

65. Conrotto, P. *et al.* Sema4D induces angiogenesis through Met recruitment by Plexin B1. *Blood* 105, 4321–4329 (2005).

References 56–65 provide information on how MET-dependent signals can be tuned by the association of MET with surface transmembrane partners, including β4 integrin, CD44 and plexin B.

66. Capparuccia, L. & Tamagnone, L. Semaphorin signaling in cancer cells and in cells of the tumor microenvironment — two sides of a coin. *J. Cell Sci.* 122, 1723–1736 (2009).

67. Johnson, G. L. & Lapadat, R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* 298, 1911–1912 (2002).

68. Stella, M. C. , Trusolino, L. , Pennacchietti, S. & Comoglio, P. M. Negative feedback regulation of Met-dependent invasive growth by Notch. *Mol. Cell. Biol.* 25, 3982–3996 (2005).

69. Lee, C. C. *et al.* Overexpression of sprouty 2 inhibits HGF/SF-mediated cell growth, invasion, migration, and cytokinesis. *Oncogene* 23, 5193–5202 (2004).

70. Rodrigues, G. A. , Park, M. & Schlessinger, J. Activation of the JNK pathway is essential for transformation by the Met oncogene. *EMBO J.* 16, 2634–2645 (1997).

71. Lamorte, L. , Kamikura, D. M. & Park, M. A switch from p130Cas/Crk to Gab1/Crk signaling correlates with anchorage independent growth and JNK activation in cells transformed by the Met receptor oncoprotein. *Oncogene* 19, 5973–5981 (2000).

72. Recio, J. A. & Merlino, G. Hepatocyte growth factor/scatter factor activates proliferation in melanoma cells through p38 MAPK, ATF-2 and cyclin D1. *Oncogene* 21, 1000–1008 (2002).

73. Rasola, A. *et al.* Hepatocyte growth factor sensitizes human ovarian carcinoma cell lines to paclitaxel and cisplatin. *Cancer Res.* 64, 1744–1750 (2004).

74. Coltella, N. *et al.* p38 MAPK turns hepatocyte growth factor to a death signal that commits ovarian cancer cells to chemotherapy-induced apoptosis. *Int. J. Cancer* 118, 2981–2990 (2006).

75. Karin, M. , Cao, Y. , Greten, F. R. & Li, Z. W. NF-κB in cancer: from innocent bystander to major culprit. *Nature Rev. Cancer* 2, 301–310 (2002).

76. Fan, S. *et al.* Role of NF-κB signaling in hepatocyte growth factor/scatter factor-mediated cell protection. *Oncogene* 24, 1749–1766 (2005).

77. Müller, M. , Morotti, A. & Ponzetto, C. Activation of NF-κB is essential for hepatocyte growth factor-mediated proliferation and tubulogenesis. *Mol. Cell. Biol.* 22, 1060–1072 (2002).

78. Scita, G. & Di Fiore, P. P. The endocytic matrix. *Nature* 463, 464–473 (2010).

79. Kermorgant, S. & Parker, P. J. c-Met signaling: spatiotemporal decisions. *Cell Cycle* 4, 352–355 (2005).

80. Liu, Z. X. , Yu, C. F. , Nickel, C. , Thomas, S. & Cantley, L. G. Hepatocyte growth factor induces ERK-dependent paxillin phosphorylation and regulates paxillin–focal adhesion kinase association. *J. Biol. Chem.* 277, 10452–10458 (2002).

81. Kermorgant, S. , Zicha, D. & Parker, P. J. PKC controls HGF-dependent c-Met traffic, signalling and cell migration. *EMBO J.* 23, 3721–3734 (2004).

82. Rosse, C. *et al.* PKC and the control of localized signal dynamics. *Nature Rev. Mol. Cell Biol.* 11, 103–112 (2010).

83. Kermorgant, S. , Zicha, D. & Parker, P. J. Protein kinase C controls microtubule-based traffic but not proteasomal degradation of c-Met. *J. Biol. Chem.* 278, 28921–28929 (2003).

84. Kermorgant, S. & Parker, P. J. Receptor trafficking controls weak signal delivery: a strategy used by c-Met for STAT3 nuclear accumulation. *J. Cell Biol.* 182, 855–863 (2008).

85. Sorkin, A. & von Zastrow, M. Endocytosis and signalling: intertwining molecular networks. *Nature Rev. Mol. Cell Biol.* 10, 609–622 (2009).

86. Peschard, P. *et al.* Mutation of the c-Cbl TKB domain binding site on the Met receptor tyrosine kinase converts it into a transforming protein. *Mol. Cell* 8, 995–1004 (2001).

87. Carter, S. , Urbé, S. & Clague, M. J. The met receptor degradation pathway: requirement for Lys48-linked polyubiquitin independent of proteasome activity. *J. Biol. Chem.* 279, 52835–52839 (2004).

88. Hammond, D. E. , Urbé, S., Vande Woude, G. F. & Clague, M. J. Down-regulation of Met, the receptor for hepatocyte growth factor. *Oncogene* 20, 2761–2770 (2001).

89. Li, N. , Xiang, G. S. , Dokainish, H. , Ireton, K. & Elferink, L. A. The *Listeria* protein internalin B mimics hepatocyte growth factor-induced receptor trafficking. *Traffic* 6, 459–473 (2005).

90. Oved, S. & Yarden, Y. Signal transduction: molecular ticket to enter cells. *Nature* 416, 133–136 (2002).

91. Petrelli, A. *et al.* The endophilin–CIN85–Cbl complex mediates ligand-dependent downregulation of c-Met. *Nature* 416, 187–190 (2002).

92. Goldoni, S. *et al.* Decorin is a novel antagonistic ligand of the Met receptor. *J. Cell Biol.* 185, 743–754 (2009).

93. Polo, S. & Di Fiore, P. P. Endocytosis conducts the cell signaling orchestra. *Cell* 124, 897–900 (2006).

94. Foveau, B. *et al.* Down-regulation of the met receptor tyrosine kinase by presenilin-dependent regulated intramembrane proteolysis. *Mol. Biol. Cell.* 20, 2495–2507 (2009).

95. Prat, M. *et al.* C-terminal truncated forms of Met, the hepatocyte growth factor receptor. *Mol. Cell. Biol.* 11, 5954–5962 (1991).

96. Galvani, A. P. , Cristiani, C. , Carpinelli, P. , Landonio, A. & Bertolero, F. Suramin modulates cellular levels of hepatocyte growth factor receptor by inducing shedding of a soluble form. *Biochem. Pharmacol.* 50, 959–966 (1995).

97. Nath, D. , Williamson, N. J. , Jarvis, R. & Murphy, G. Shedding of c-Met is regulated by crosstalk between a G-protein coupled receptor and the EGF receptor and is mediated by a TIMP-3 sensitive metalloproteinase. *J. Cell Sci.* 114, 1213–1220 (2001).

98. Wajih, N. , Walter, J. & Sane, D. C. Vascular origin of a soluble truncated form of the hepatocyte growth factor receptor (c-met). *Circ. Res.* 90, 46–52 (2002).

99. Petrelli, A. *et al.* Ab-induced ectodomain shedding mediates hepatocyte growth factor receptor down-regulation and hampers biological activity. *Proc. Natl Acad. Sci. USA* 103, 5090–5095 (2006).

100. Schelter, F. *et al.* A disintegrin and metalloproteinase-10 (ADAM-10) mediates DN30 antibody-induced shedding of the met surface receptor. *J. Biol. Chem.* 285, 26335–26340 (2010).

101. Vigna, E. *et al.* "Active" cancer immunotherapy by anti-Met antibody gene transfer. *Cancer Res.* 68, 9176–9183 (2008).

102. Pacchiana, G. *et al.* Monovalency unleashes the full therapeutic potential of the DN-30 anti-Met antibody. *J. Biol. Chem.* 285, 36149–36157 (2010).

103. Michieli, P. *et al.* Targeting the tumor and its microenvironment by a dual-function decoy Met receptor. *Cancer Cell* 6, 61–73 (2004).

104. Zhang, Y. W. , Graveel, C. , Shinomiya, N. & Vande Woude, G. F. Met decoys: will cancer take the bait? *Cancer Cell* 6, 5–6 (2004).

105. Barabasi, A.-L. & Oltavi, Z. N. Network biology: understanding the cell's functional organization. *Nature Rev. Genet.* 5, 101–113 (2004).

106. Schmidt, C. *et al.* Scatter factor/hepatocyte growth factor is essential for liver development. *Nature* 373, 699–702 (1995).

107. Uehara, Y. *et al.* Placental defect and embryonal lethality in mice lacking hepatocyte growth factor/scatter factor. *Nature* 373, 702–705 (1995).

108. Bladt, F. , Riethmacher, D. , Isenmann, S. , Aguzzi, A. & Birchmeier, C. Essential role for the c-*met* receptor in the migration of myogenic precursor cells into the limb bud. *Nature* 376, 768–771 (1995).

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

110. Maina, F. , Hilton, M. C. , Ponzetto, C. , Davies, A. M. & Klein, R. Met receptor signaling is required for sensory nerve development and HGF promotes axonal growth and survival of sensory neurons. *Genes Dev.* 11, 3341–3350 (1997).

111. Maina, F. *et al.* Multiple roles for hepatocyte growth factor in sympathetic neuron development. *Neuron* 20, 835–846 (1998).

112. Helmbacher, F. *et al.* Met signaling is required for recruitment of motor neurons to PEA3-positive motor neurons. *Neuron* 39, 767–777 (2003).

113. Maina, F. *et al.* Coupling Met to specific pathways results in distinct developmental outcomes. *Mol. Cell* 7, 1293–1306 (2001). This article addresses *in vivo* how activation of individual MET-dependent signalling pathways influences the execution of specific biological responses.

114. Sachs, M. *et al.* Essential role of Gab1 for signaling by the c-Met receptor *in vivo*. *J. Cell Biol.* 150, 1375–1384 (2000).

115. Schaeper, U. *et al.* Distinct requirements for Gab1 in Met and EGF receptor signaling *in vivo*. *Proc. Natl Acad. Sci. USA* 104, 15376–15381 (2007). The authors show that the multi-adaptor GAB1 promotes different biological outcomes *in vivo* through the recruitment of distinct effectors.

116. Debnath, J. & Brugge, J. S. Modelling glandular epithelial cancers in three-dimensional cultures. *Nature Rev. Cancer* 5, 675–688 (2005).

117. Rosário, M. & Birchmeier, W. How to make tubes: signaling by the Met receptor tyrosine kinase. *Trends Cell Biol.* 13, 328–335 (2003).

118. O'Brien, L. E. *et al.* ERK and MMPs sequentially regulate distinct stages of epithelial tubule development. *Dev. Cell* 7, 21–32 (2004). A mechanistic dissection, using three-dimensional culture models, of the signalling pathways that mediate the sequential steps of MET-induced epithelial tubulogenesis.

119. Rosário, M. & Birchmeier, W. Making tubes: step by step. *Dev. Cell* 7, 3–5 (2004).

120. Leroy, P. & Mostov, K. E. Slug is required for cell survival during partial epithelial-mesenchymal transition of HGF-induced tubulogenesis. *Mol. Biol. Cell* 18, 1943–1952 (2007).

121. Rosenthal, E. L. *et al.* Role of the plasminogen activator and matrix metalloproteinase systems in epidermal growth factor- and scatter factor-stimulated invasion of carcinoma cells. *Cancer Res.* 58, 5221–5230 (1998).

122. Nabeshima, K. *et al.* Front-cell-specific expression of membrane-type 1 matrix metalloproteinase and gelatinase A during cohort migration of colon carcinoma cells induced by hepatocyte growth factor/scatter factor. *Cancer Res.* 60, 3364–3369 (2000).

123. Monvoisin, A. *et al.* Involvement of matrix metalloproteinase type-3 in hepatocyte growth factor-induced invasion of human hepatocellular carcinoma cells. *Int. J. Cancer* 97, 157–162 (2002).

124. Miyazawa, K. *et al.* Molecular cloning and sequence analysis of cDNA for human hepatocyte growth factor. *Biochem. Biophys. Res. Commun.* 163, 967–973 (1989).

125. Huh, C. G. *et al.* Hepatocyte growth factor/c-*met* signaling pathway is required for efficient liver regeneration and repair. *Proc. Natl Acad. Sci. USA* 101, 4477–4482 (2004).

126. Borowiak, M. *et al.* Met provides essential signals for liver regeneration. *Proc. Natl Acad. Sci. USA* 101, 10608–10613 (2004). References 125 and 126 describe the first genetic demonstrations that MET is essential in the regeneration of adult liver.

127. Bard-Chapeau, E. A. *et al.* Concerted functions of Gab1 and Shp2 in liver regeneration and hepatoprotection. *Mol. Cell. Biol.* 26, 4664–4674 (2006).

128. Liu, Y. Hepatocyte growth factor in kidney fibrosis: therapeutic potential and mechanisms of action. *Am. J. Physiol. Renal Physiol.* 287, F7–F16 (2004).

129. Wynn, T. A. Cellular and molecular mechanisms of fibrosis. *J. Pathol.* 214, 199–210 (2008).

130. Ueki, T. *et al.* Hepatocyte growth factor gene therapy of liver cirrhosis in rats. *Nature Med.* 5, 226–230 (1999).

131. Yang, J. , Dai, C. & Liu, Y. Hepatocyte growth factor suppresses renal interstitial myofibroblast activation and intercepts Smad signal transduction. *Am. J. Pathol.* 163, 621–632 (2003).

132. Dai, C. & Liu, Y. Hepatocyte growth factor antagonizes the profibrotic action of TGF-β1 in mesangial cells by stabilizing Smad transcriptional corepressor TGIF. *J. Am. Soc. Nephrol.* 15, 1402–1412 (2004).

133. Kim, W. H. , Matsumoto, K. , Bessho, K. & Nakamura, T. Growth inhibition and apoptosis in liver myofibroblasts promoted by hepatocyte growth factor leads to resolution from liver cirrhosis. *Am. J. Pathol.* 166, 1017–1028 (2005).

134. Blanpain, C. & Fuchs, E. Epidermal homeostasis: a balancing act of stem cells in the skin. *Nature Rev. Mol. Cell Biol.* 10, 207–217 (2009).

135. Chmielowiec, J. *et al.* c-Met is essential for wound healing in the skin. *J. Cell Biol.* 177, 151–162 (2007).

136. Pennacchietti, S. *et al.* Hypoxia promotes invasive growth by transcriptional activation of the *met* protooncogene. *Cancer Cell* 3, 347–361 (2003).

137. Giordano, S. *et al.* A point mutation in the *MET* oncogene abrogates metastasis without affecting transformation. *Proc. Natl Acad. Sci. USA* 94, 13868–13872 (1997).

138. Bardelli, A. *et al.* Concomitant activation of pathways downstream of Grb2 and PI3-kinase is required for *MET*-mediated metastasis. *Oncogene* 18, 1139–1146 (1999).

139. Ma, P. C. *et al.* Downstream signalling and specific inhibition of c-Met/HGF pathway in small cell lung cancer: implications for tumour invasion. *Br. J. Cancer* 97, 368–377 (2007).

140. Schmidt, L. *et al.* Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. *Nature Genet.* 16, 68–73 (1997).

141. Stella, G. M. *et al.* *MET* mutations in cancers of unknown primary origin. *Hum. Mutat.* 14 Oct 2010 (doi: 10.1002/humu.21374).

142. Smolen, G. A. *et al.* Amplification of *MET* may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752. *Proc. Natl Acad. Sci. USA* 103, 2316–2321 (2006).

143. McDermott, U. *et al.* Identification of genotype-correlated sensitivity to selective kinase inhibitors by using high-throughput tumor cell line profiling. *Proc. Natl Acad. Sci. USA* 104, 19936–19941 (2007). References 142 and 143 are the first demonstration that only cell lines with amplification of the *Met* gene respond to MET inhibition with substantial growth impairment.

144. Guo, A. *et al.* Signaling networks assembled by oncogenic EGFR and c-Met. *Proc. Natl Acad. Sci. USA* 105, 692–697 (2008).

145. Bertotti, A. *et al.* Only a subset of Met-activated pathways are required to sustain oncogene addiction. *Sci. Signal.* 2, ra80 (2009). References 144 and 145 are the first large-scale analyses of the signalling and transcriptional consequences of MET inhibition in drug-sensitive, MET-addicted cell lines.

146. Bertotti, A. *et al.* Inhibition of Src impairs the growth of Met-addicted gastric tumors. *Clin. Cancer Res.* 16, 3933–3943 (2010).

147. Amit, I. *et al.* A module of negative feedback regulators defines growth factor signaling. *Nature Genet.* 39, 503–512 (2007).

148. Jeffrey, K. L. , Camps, M. , Rommel, C. & Mackay, C. R. Targeting dual-specificity phosphatases: manipulating MAP kinase signalling and immune responses. *Nature Rev. Drug Discov.* 6, 391–403 (2007).

149. Kim, S. *et al.* MicroRNA *mir199-a* regulates the *MET* proto-oncogene and the downstream extracellular signal-regulated kinase 2 (*ERK2*). *J. Biol. Chem.* 283, 18158–18166 (2008).

150. Migliore, C. *et al.* MicroRNAs impair MET-mediated invasive growth. *Cancer Res.* 68, 10128–10136 (2008).

151. Taulli, R. *et al.* The muscle-specific microRNA miR-206 blocks human rhabdomyosarcoma growth in xenotransplanted mice by promoting myogenic differentiation. *J. Clin. Invest.* 119, 2366–2378 (2009).
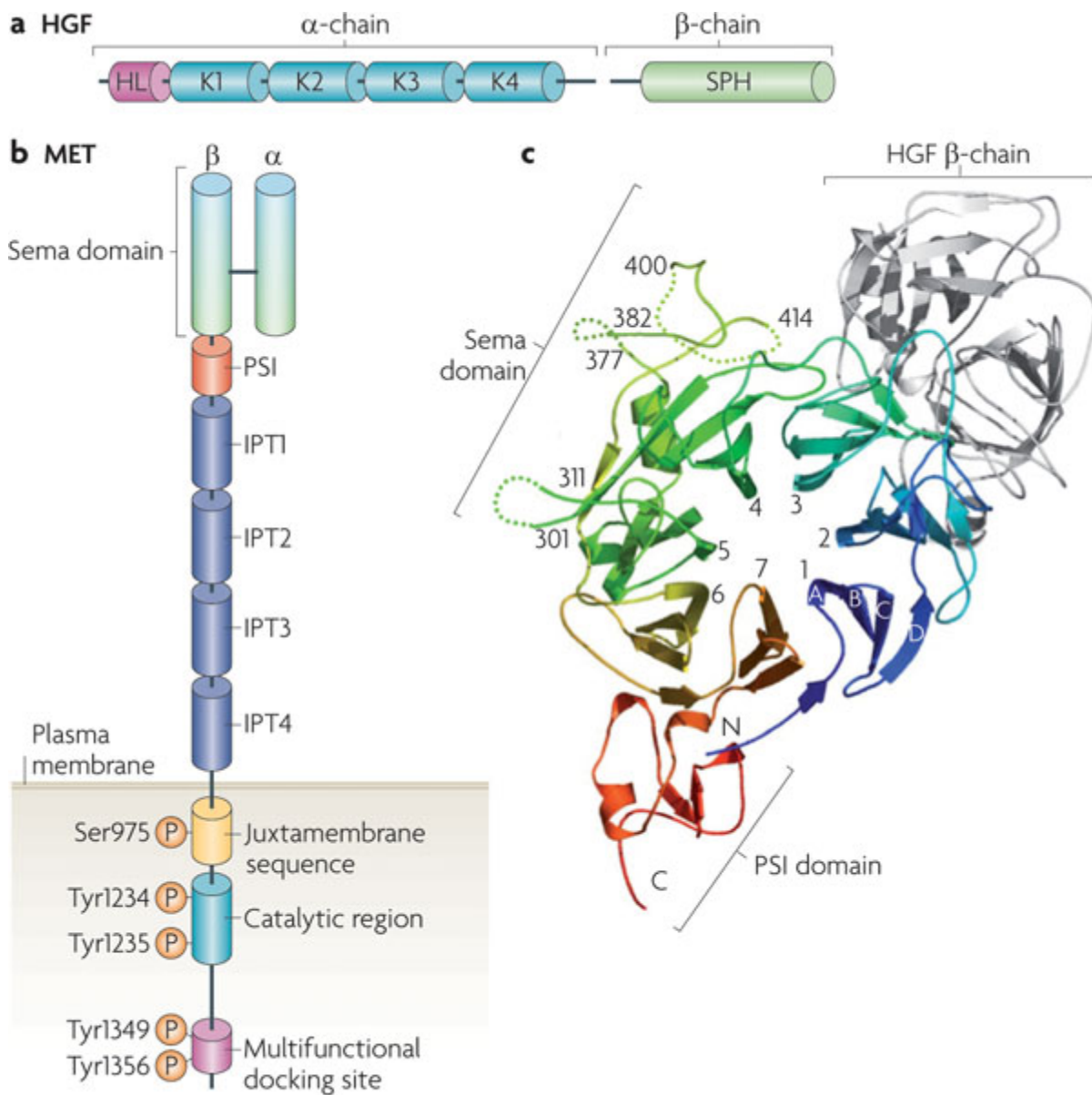
152. Garofalo, M. *et al. miR-221&222* regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. *Cancer Cell* 16, 498–509 (2009).

153. Fornari, F. *et al.* MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. *Cancer Res.* 70, 5184–5193 (2010).

154. Bradbury, J. A two-pronged approach to the clinical use of HGF. *Lancet* 351, 272 (1998).

155. Basilico, C. , Arnesano, A. , Galluzzo, M. , Comoglio, P. M. & Michieli, P. A high affinity hepatocyte growth factor-binding site in the immunoglobulin-like region of Met. *J. Biol. Chem.* 283, 21267–21277 (2008).

156. Stamos, J. , Lazarus, R. A. , Yao, X. , Kirchhofer, D. & Wiesmann, C. Crystal structure of the HGF β-chain in complex with the Sema domain of the Met receptor. *EMBO J.* 23, 2325–2335 (2004).

157. Shima, N. *et al.* Tumor cytotoxic factor/hepatocyte growth factor from human fibroblasts: cloning of its cDNA, purification and characterization of recombinant protein. *Biochem. Biophys. Res. Commun.* 180, 1151–1158 (1991).

158. Matteucci, E. , Castoldi, R. & Desiderio, M. A. Hepatocyte growth factor induces pro-apoptotic genes in HepG2 hepatoma but not in B16-F1 melanoma cells. *J. Cell. Physiol.* 186, 387–396 (2001).

159. Mizuno, S. , Matsumoto, K. & Nakamura, T. HGF reduces advancing lung fibrosis in mice: a potential role for MMP-dependent myofibroblast apoptosis. *FASEB J.* 19, 580–582 (2005).

160. Tacchini, L. , De Ponti, C. , Matteucci, E. , Follis, R. & Desiderio, M. A. Hepatocyte growth factor-activated NF-κB regulates HIF-1 activity and ODC expression, implicated in survival, differently in different carcinoma cell lines. *Carcinogenesis* 25, 2089–2100 (2004).

161. Wang, X. *et al.* A mechanism of cell survival: sequestration of Fas by the HGF receptor Met. *Mol. Cell* 9, 411–421 (2002).

162. Tulasne, D. *et al.* Proapoptotic function of the Met tyrosine kinase receptor through caspase cleavage. *Mol. Cell. Biol.* 24, 10328–10339 (2004).

163. Foveau, B. *et al.* Amplification of apoptosis through sequential caspase cleavage of the Met tyrosine kinase receptor. *Cell Death Differ.* 14, 752–764 (2007).

164. Potempa, S. & Ridley, A. J. Activation of both MAP kinase and phosphatidylinositide 3-kinase by Ras is required for hepatocyte growth factor/scatter factor-induced adherens junction disassembly. *Mol. Biol. Cell* 9, 2185–2200 (1998).

165. Rahimi, N. , Hung, W. , Tremblay, E. , Saulnier, R. & Elliott, B. c-Src kinase activity is required for hepatocyte growth factor-induced motility and anchorage-independent growth of mammary carcinoma cells. *J. Biol. Chem.* 273, 33714–33721 (1998).

166. Royal, I. & Park, M. Hepatocyte growth factor-induced scatter of Madin–Darby canine kidney cells requires phosphatidylinositol 3-kinase. *J. Biol. Chem.* 270, 27780–27787 (1995).

167. Khwaja, A. , Lehmann, K. , Marte, B. M. & Downward, J. Phosphoinositide 3-kinase induces scattering and tubulogenesis in epithelial cells through a novel pathway. *J. Biol. Chem.* 273, 18793–18801 (1998).

168. Sakkab, D. *et al.* Signaling of hepatocyte growth factor/scatter factor (HGF) to the small GTPase Rap1 via the large docking protein Gab1 and the adapter protein CRKL. *J. Biol. Chem.* 275, 10772–10778 (2000).

169. Xiao, G. H. *et al.* Anti-apoptotic signaling by hepatocyte growth factor/Met via the phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase pathways. *Proc. Natl Acad. Sci. USA* 98, 247–252 (2001).

Hepatocyte growth factor (HGF; also known as scatter factor) is similar to plasminogen, a circulating zymogen that promotes the dissolution of fibrin blood clots in its active form as the serine protease plasmin. Both HGF and plasminogen are synthesized as a single-chain precursor and then converted into an active α- and β-chain heterodimer by extracellular proteases. HGF consists of six domains: an amino-terminal hairpin loop (HL), four kringle domains (K1–K4; each defined by three conserved disulphide bonds) and a serine protease homology (SPH) domain that lacks proteolytic activity (see the figure, part **a**).

The HGF receptor MET is a single-pass heterodimer comprising an entirely extracellular α-subunit that is linked by a disulphide bond to a transmembrane β-subunit, which contains the intracellular catalytic activity (see the figure, part **b**). The extracellular region of MET includes three functional domains: the Sema domain (which is also found in the semaphorins and plexins) spans the first 500 residues at the N terminus, encompassing the whole α-subunit and part of the β-subunit; the PSI domain (which is also present in the plexins, semaphorins and integrins, hence its name) covers approximately 50 residues and contains four conserved disulphide bonds; the residual 400 residues connecting the PSI domain to the transmembrane helix are organized into four IPT (immunoglobulin-like fold shared by plexins and transcriptional factors) domains. The intracellular segment is composed of three portions: a juxtamembrane sequence that downregulates kinase activity following phosphorylation of Ser975; a catalytic region that positively modulates kinase activity following *trans*-phosphorylation of Tyr1234 and Tyr1235; and a carboxy-terminal multifunctional docking site that contains two docking tyrosines (Tyr1349 and Tyr1356) that are involved in the recruitment of several transducers and adaptors.

HGF contains two MET binding sites that have different affinities. The high-affinity site is located in the α-chain and recognizes the IPT3 and IPT4 domains of MET independently of HGF processing and maturation[155]. The low-affinity site lies within the β-chain, is exposed only after HGF activation and interacts with the Sema domain of MET[156]. This latter association is depicted as a ribbon representation (see the figure, part **c**). The HGF β-chain is shown in grey and part of the extracellular region of MET is shown in a gradient of rainbow colours, from the N terminus (shown in blue) to the PSI domain (shown in red). The numbers in the centre refer to the blades, with the β-strands in blade 1 labelledA–D. The numbers on the edge of the Sema domain represent residue numbers on each side of a disordered region (dotted line). Part c modified, with permission, from Ref. 156 © (2004) Macmillan Publishers Ltd. All rights reserved.
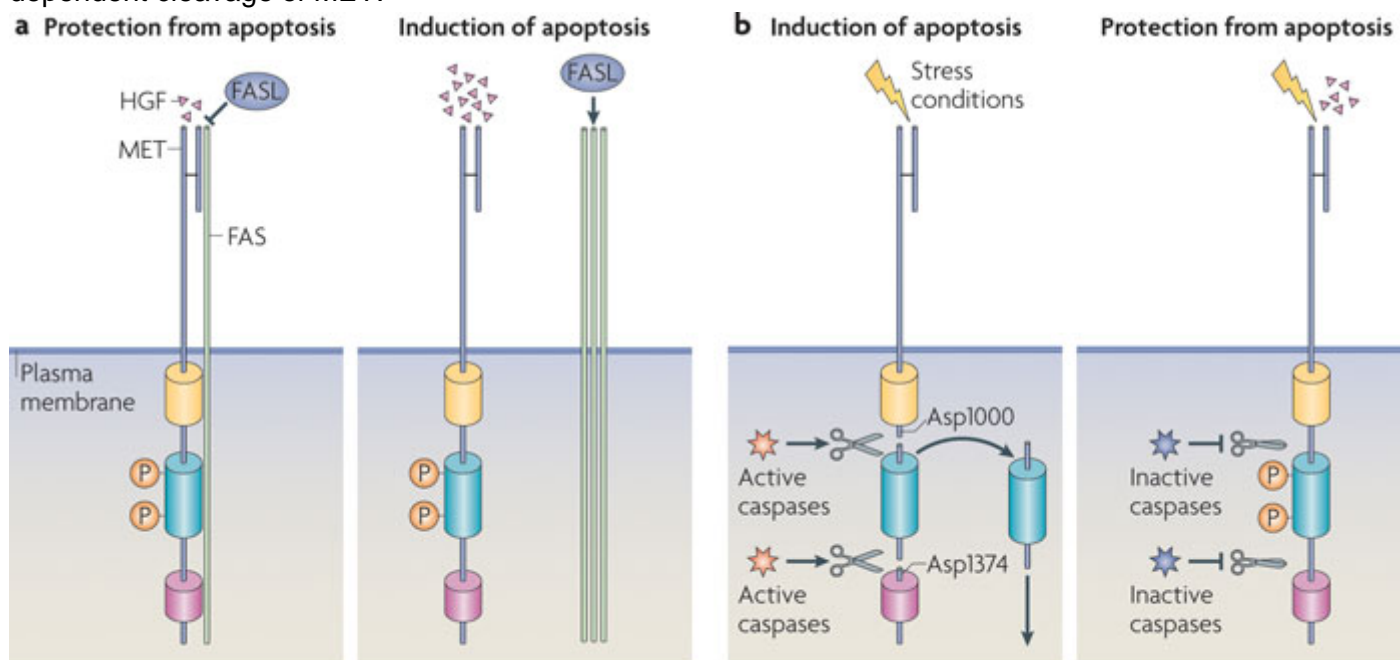
a HGF

α-chain — K1, K2, K3, K4; HL
β-chain — SPH

b MET

β   α
Sema domain
PSI
IPT1
IPT2
IPT3
IPT4
Plasma membrane
Ser975 (P) — Juxtamembrane sequence
Tyr1234 (P) — Catalytic region
Tyr1235 (P)
Tyr1349 (P) — Multifunctional docking site
Tyr1356 (P)

c

HGF β-chain
Sema domain
400
382
414
377
311
301
4 3
5 2
7 1
6 A B C D
N
PSI domain
C

BOX 2

Despite acting as a prototypical survival factor, hepatocyte growth factor (HGF; also known as scatter factor) was initially named 'tumour cytotoxic factor' for its ability to elicit apoptotic responses in sarcoma[157] and hepatoma[158] cells. These results were confirmed in other cell types, including liver and lung myofibroblasts[133, 159] and breast carcinoma cell lines[160], in which HGF-induced apoptosis was attributed to the activation of protein kinase C and Jun amino-terminal kinase (JNK) or to the induction of reactive oxygen species. In ovarian cancer cells that have been treated with conventional chemotherapeutics, HGF enhances apoptosis through a p38 mitogen-activated protein kinase (MAPK)-dependent pathway[73, 74].

The mechanism by which MET (also known as HGF receptor) activation leads to opposite outcomes — survival by default and apoptosis in restricted cellular contexts — has been only partially elucidated. The extracellular domain of MET interacts with the death receptor FAS (also known as CD95 and TNFRSF6), which prevents FAS receptor–FAS ligand (FASL) recognition and FAS self-aggregation, therefore limiting apoptosis through the extrinsic pathway[161](see the figure, part **a**). However, elevated HGF levels sensitize cells to FASL-mediated apoptosis[161]. It has been proposed that excessively high concentrations of HGF would titre out MET and liberate FAS from its association with MET, suggesting that the pro-apoptotic effects evoked by HGF in some cell lines could be related to a critical stoichiometry in the reciprocal amounts of HGF, MET, FAS and FASL.

The intracellular domain of MET is also involved in the positive control of apoptosis. Under stress conditions, the MET cytoplasmic portion undergoes sequential cleavage by caspases, first at an aspartic acid residue in the carboxy-terminal end (Asp1374) and then at a second aspartic acid in the juxtamembrane portion (Asp1000) (see the figure, part **b**). This yields an intracellular soluble fragment, which is comprised of the kinase domain and triggers cell death[162, 163]. Notably, if stress induction is accompanied by HGF stimulation, caspase activation is reduced and generation of the intracellular pro-apoptotic fragment is prevented[162]. This points to the presence of a cellular rheostat, in which life or death are ultimately dictated by the anti-apoptotic input that is generated by HGF-dependent activation of MET and the pro-apoptotic input that is generated by caspase-dependent cleavage of MET.
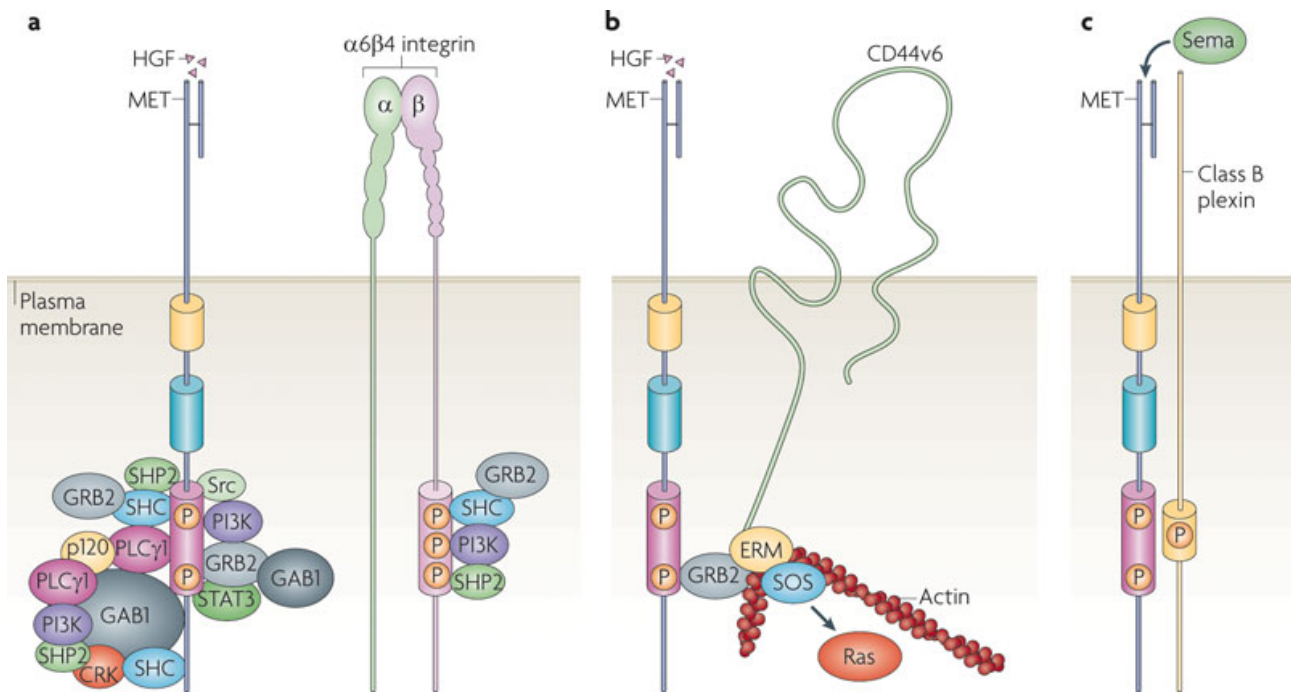

a Protection from apoptosis / Induction of apoptosis / **b** Induction of apoptosis / Protection from apoptosis

Figure 1. The strength, duration and versatility of signals triggered by MET (also known as HGF receptor) are regulated by a network of signalling amplifiers and co-receptors that physically associate with MET. **a** | Adaptor proteins that have a scaffolding function, such as GRB2-associated-binding protein 1 (GAB1) and the α6β4 integrin, associate with MET and act as supplementary docking platforms for the further binding of signal transducers, thus enhancing signalling outputs. GAB1 interacts with MET both indirectly (through growth factor receptor-bound protein 2 (GRB2)) and directly. Following MET-dependent tyrosine phosphorylation, GAB1 provides additional sites for the recruitment of Src-homology-2 domain-containing transforming protein (SHC), phosphoinositide 3-kinase (PI3K), SHP2 (also known as PTPN11), CRK, phospholipase Cγ1 (PLCγ1) and p120 Ras-GTPase-activating protein (p120). Similarly, the β4 subunit of the α6β4 integrin directly associates with MET, which tyrosine-phosphorylates β4 and therefore generates extra binding sites for SHC, PI3K and SHP2. GRB2 can also associate with SHC that is bound to MET or α6β4 integrin. **b** | The extracellular domain of the v6 splice variant of the hyaluronan receptor CD44 (CD44v6) forms a ternary complex with MET and hepatocyte growth factor (HGF), an event that is necessary for MET activation. The intracellular portion of CD44v6 links the MET cytoplasmic domain to actin microfilaments through GRB2 and intermediate ezrin, radixin and moesin (ERM) proteins, which facilitates MET-induced activation of Ras by the guanine nucleotide exchange factor son of sevenless (SOS). **c** | Members of the class B plexins family associate with MET and transactivate it in response to their semaphorin ligands (Sema), even in the absence of HGF, providing an alternative way to stimulate MET-driven biological responses. Semaphorin-dependent activation of MET also results in tyrosine phosphorylation of the plexin cytoplasmic domain, but the functional consequences of this event are unknown. STAT3, signal transducer and activator of transcription 3.
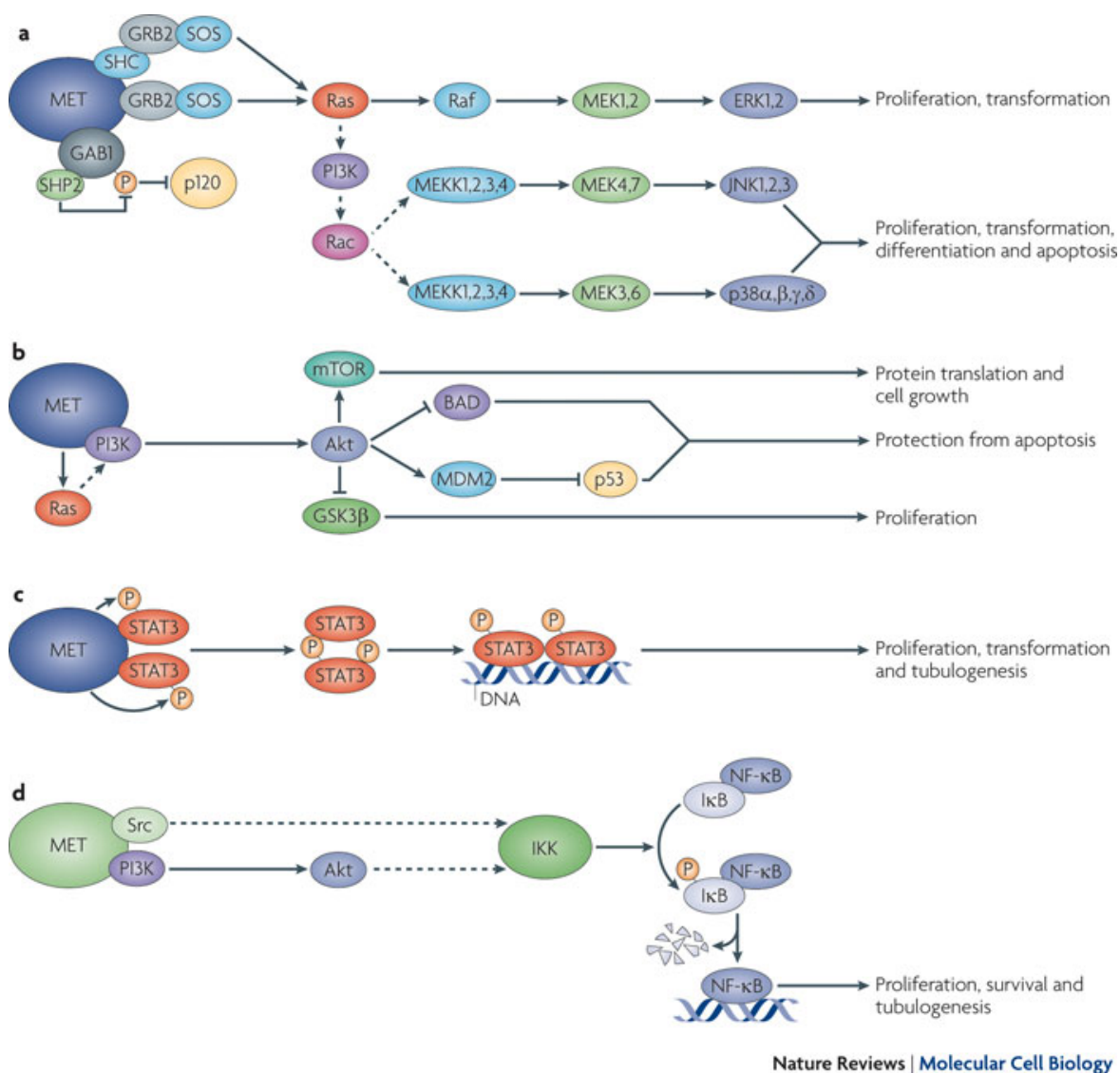
Figure 2. MET (also known as HGF receptor) triggers several downstream pathways. **a** | The mitogen-activated protein kinase (MAPK) cascades consist of three subfamilies, each of which comprises three protein kinases that activate one another sequentially. The proximal elements are activated directly or indirectly by the Ras small GTPase, which in turn is switched on by son of sevenless (SOS) and switched off by GTPase activating proteins (GAPs), such as p120 Ras-GAP (p120). SOS can be activated and p120 can be inhibited in response to MET signalling. The terminal effectors include extracellular signal-regulated kinases (ERKs), Jun amino-terminal kinases (JNKs) and p38s. These translocate to the nucleus, where they influence the activity of various transcription factors. **b** | Phosphoinositide 3-kinase (PI3K) is a lipid kinase that associates with the multifunctional docking site of MET and catalyses the formation of phosphatidylinositol (3,4,5)-triphosphate (PtdIns(3,4,5)P_3). Production of PtdIns(3,4,5)P_3 creates a docking site for Akt. Once compartmentalized at the inner side of the plasma membrane, Akt becomes activated and phosphorylates several substrates involved in cell proliferation, survival and the regulation of cell size. **c** | Signal transducer and activator of transcription 3 (STAT3) monomers bind to MET through their Src-homology-2 domain (SH2 domain) and become *trans*-phosphorylated. Thereafter, each STAT3 moiety homodimerizes, using its SH2 domain to recognize the phosphotyrosine of its partner, and translocates to the nucleus to operate as a transcription factor. **d** | In response to MET stimulation and the ensuing activation of PI3K- and Src-dependent pathways, nuclear factor-κB inhibitor-α kinase (IKK) is activated, and phosphorylates the nuclear factor-κB inhibitor-α (IκB) proteins (which are bound to nuclear factor-κB (NF-κB)). This triggers the ubiquitin-dependent degradation of IκB, resulting in the nuclear translocation of NF-κB and transcription. In all diagrams, dashed lines indicate that the signalling intermediates between the two nodes have not been characterized in detail. BAD, BCL-2 antagonist of cell death; GAB1, GRB2-associated-binding protein 1; GRB2, growth factor receptor-bound protein 2; GSK3β, glycogen synthase kinase 3β; MEK, MAPK/ERK kinase (also known as MAPKK); MEKK, MEK kinase; mTOR, mammalian target of rapamycin; SHC, SH2 domain-containing transforming protein.
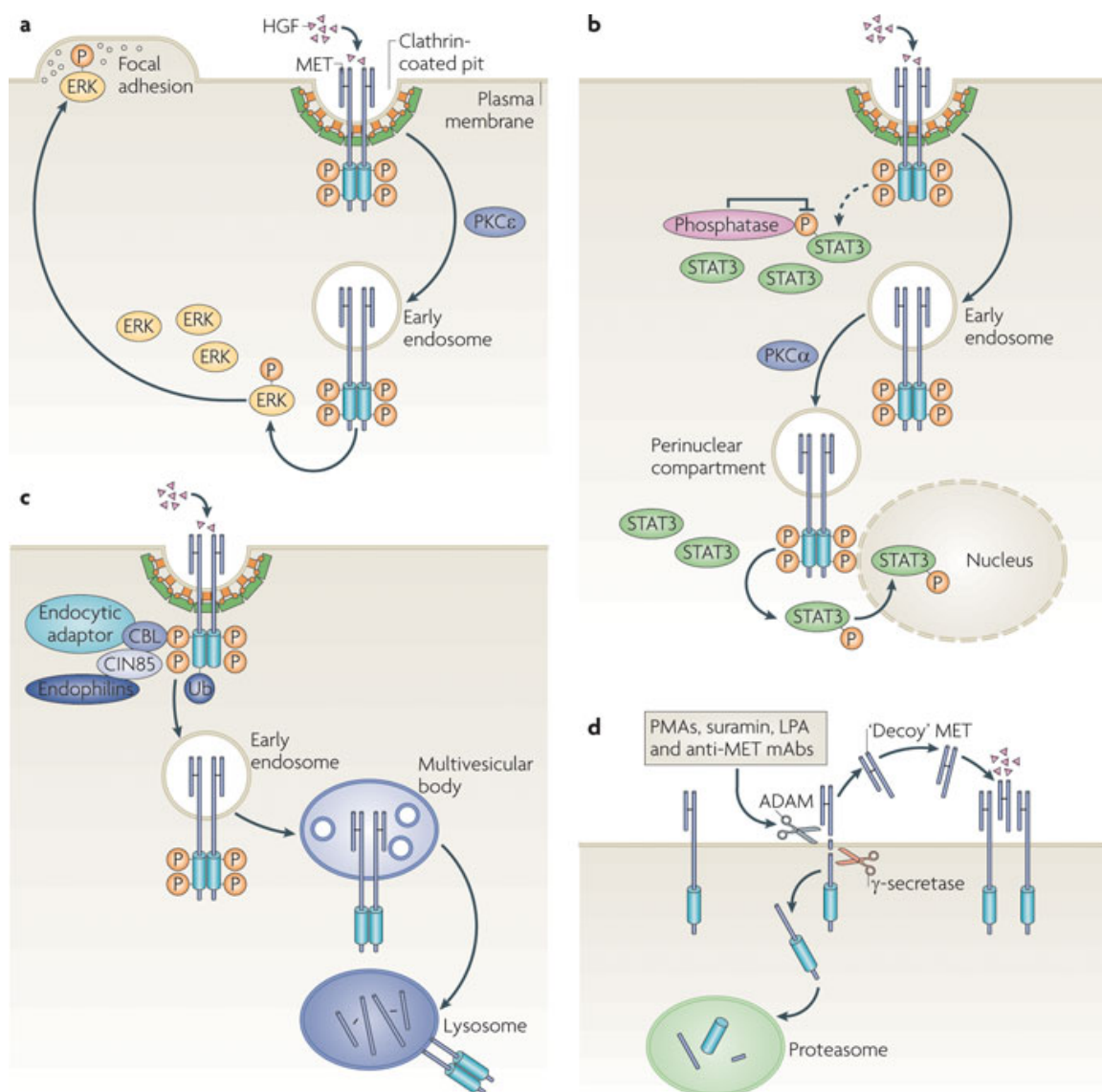
Figure 3. After being internalized from the cell surface, MET (also known as HGF receptor) can maintain a signalling-competent modality or can be demolished. **a** | The recruitment of MET into early endosomes is mediated by protein kinase Cε (PKCε) and favours the delivery of active extracellular signal-regulated kinase (ERK) to focal adhesions, where it can mediate hepatocyte growth factor (HGF; also known as scatter factor)-induced cell migration. **b** | MET sorting into non-degradative perinuclear endomembrane compartments is mediated by PKCα. In these compartments, the activity of phosphotyrosine phosphatases, which work on signal transducer and activator of transcription 3 (STAT3) to dissipate signal intensity, is low. This favours efficient phosphorylation of STAT3 by MET and translocation of STAT3 into the nucleus. **c** | Downregulation of active MET is initiated by MET association with casitas B-lineage lymphoma (CBL), an E3 ubiquitin (Ub)-protein ligase that bridges MET and endocytic adaptors. CBL also associates with endophilins through the scaffold molecule CBL-interacting protein 85 (CIN85), which promotes changes in the plasma membrane that are necessary for cell surface curvature in the early phases of endocytosis. Following endocytosis and intermediate accumulation in multivesicular bodies, MET is subjected to lysosomal degradation. **d** | MET can also undergo sequential proteolytic cleavage at two juxtamembrane sites, one in the extracellular domain and the other in the intracellular domain. The first cleavage (known as shedding), which is carried out by a disintegrin and metalloprotease (ADAM)-like extracellular protease, generates a soluble extracellular 'decoy' fragment that interferes with the activity of the full-length receptor and sequesters the ligand. This shedding is stimulated by various agents such as phorbol esters (PMAs), suramin, lysophosphatidic acid (LPA) and monoclonal antibodies (mAbs) against the MET extracellular domain. The second cleavage is performed by γ-secretase and yields an intracellular fragment that is destroyed by the proteasome in an ubiquitin-independent manner. Dashed lines indicate that the signalling intermediates between the two nodes have not been characterized in detail.
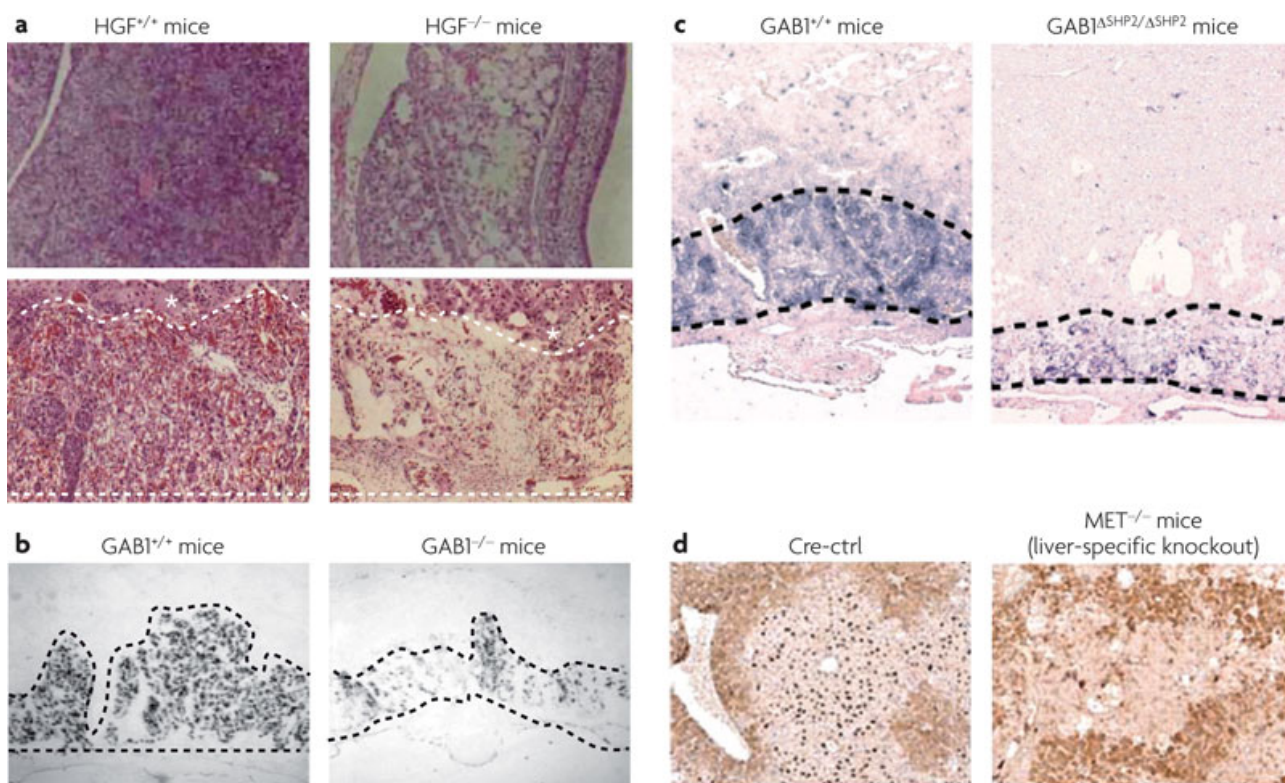
Figure 4. Mutant mice with targeted disruption of hepatocyte growth factor (HGF; also known as scatter factor)- or MET (also known as HGF receptor)-dependent signals show various abnormalities that are related to proliferative impairment. **a** | The liver of HGF knockout (HGF$^{-/-}$) mice features severe loss of cellularity and enlarged sinusoids compared with that of wild-type embryos (HGF$^{+/+}$ mice). Similarly, the labyrinth layer (delimited by dotted lines) contains fewer trophoblast cells, embryonic vessels and maternal sinuses and more mesenchymal cells. The junctional zone (denoted by an asterisk) lies above the labyrinth. **b** | An analogous placental defect, with a large reduction in the size of the labyrinth layer, occurs in mice with genetic deletion of GRB2-associated-binding protein 1 (GAB1). **c** | The same phenotype is also displayed in mice that harbour a knock-in point mutation that abolishes GAB1 interaction with SHP2 (also known as PTPN11) (GAB1$^{\Delta SHP2/\Delta SHP2}$ mice). **d** | In adult animals, Cre–*loxP*-mediated conditional deletion of MET in the liver impairs hepatic regeneration following toxic insult, as shown by the reduction of hepatocytes incorporating bromo-deoxyuridine (BrdU), which indicates a decrease in proliferating hepatocytes. Larger necrotic areas are also evident in the liver of MET$^{-/-}$ mice compared with control liver (Cre-ctrl). A similar decrease of proliferating hepatocytes can be observed during post-hepatectomy regeneration in mice with liver-specific knockout of GAB1 and in mice with liver-targeted disruption of SHP2 (Ref. 127). Images in part **a** modified, with permission, from Refs 106, 107 © (1995) Macmillan Publishers Ltd. All rights reserved. Images in part **b** modified, with permission, from Ref. 114 © (2000) The Rockefeller University Press. Images in part **c** modified, with permission, from Ref. 115 © (2007) National Academy of Sciences, USA. Images in part **d** modified, with permission, from Ref. 125 © (2004) National Academy of Sciences, USA.