The role of the HGF/Met axis in mesothelioma

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Abstract

Malignant mesothelioma is an asbestos-related cancer that occurs most commonly in the pleural space and is incurable. Increasing evidence suggests that aberrant receptor tyrosine kinase (RTK)-directed signalling plays a key role in the pathogenesis of this cancer. In the majority of mesotheliomas, up-regulated expression or signalling by Met, the receptor for hepatocyte growth factor (HGF) can be demonstrated. Following binding of ligand, Met relays signals that promote cell survival, proliferation, movement, invasiveness, branching morphogenesis and angiogenesis. Here we describe the HGF/Met axis and review the mechanisms that lead to the aberrant activation of this signalling system in mesothelioma. We also describe the cross-talk that occurs between HGF/Met and a number of other receptors, ligands and co-receptor systems. The prevalent occurrence of HGF/Met dysregulation in patients with mesothelioma sets the scene for the investigation of pharmaceutical inhibitors of this axis. In light of the inter-relationship between HGF/Met and other ligand receptor, combinatorial targeting strategies may provide opportunities for therapeutic advancement in this challenging tumour.

Malignant mesothelioma

Malignant mesothelioma is a cancer that arises from the mesothelial cells lining the body cavities and is associated with high morbidity and mortality. In approximately 90 % of cases, the disorder occurs in the pleural space, though mesothelioma may also originate within the peritoneal cavity and pericardium.

The primary causative agent associated with the development of malignant mesothelioma is inhalation of asbestos fibres (80 % attributable fraction). Industrial use of asbestos was widespread during the 20th century and is still ubiquitously used in some developing countries. There is a long latent period between asbestos exposure and the development of mesothelioma (average 42.8 years) [1]. Consequently, the incidence of mesothelioma continues to rise within Europe [2]. Cancer risk is greatest following exposure to the needle-like asbestos fibres that constitute the amphiboles, best exemplified by blue (crocidolite) and brown (amosite) asbestos. Other risk factors for mesothelioma are less well understood but include underlying genetic background, exposure to radiation and non-asbestos mineral fibres, notably erionite. Prior infection with the DNA tumour virus, SV40 (simian virus 40), has also been implicated in disease development [1].

Three major histological subtypes of mesothelioma have been described: epithelioid, sarcomatoid and biphasic. The epithelioid variant is most common (>50 % in most series) and has a slightly better prognosis compared with tumours with any sarcomatoid features. Biphasic tumours comprise at least 10 % of both epithelioid and sarcomatoid elements, as determined microscopically. Definitive diagnosis of malignant mesothelioma can be challenging and immunohistochemistry is usually required to differentiate this cancer from other pleural malignant tumours (e.g. metastatic lung or breast adenocarcinoma) and reactive mesothelial proliferations.

The management of malignant mesothelioma remains controversial. Radical surgery, as part of multi-modality therapy, has been promoted but the incidence of post-operative morbidity is high and survival benefit remains unproven. Palliative chemotherapy using anti-folate agents (pemetrexed or raltitrexed) in combination with cisplatin has prolonged survival by a few months in suitable patients [3] – however, only a minority of patients respond well to such therapy. Irrespective of management regimen, long-term control of malignant mesothelioma remains elusive and prognosis is very poor with a median survival from presentation of 9–12 months [4].

Key words: hepatocyte growth factor, malignant mesothelioma, Met, receptor tyrosine kinase, scatter factor.

Abbreviations: EGFR, epidermal growth factor receptors; Fra-1, fos-related antigen-1; HGF, hepatocyte growth factor; Met, mesenchymal epithelial transition factor; MPM, malignant pleural mesothelioma; MSP, macrophage stimulating protein; RTK, receptor tyrosine kinases; VEGFR, vascular endothelial growth factor receptor.

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Molecular pathogenesis of malignant mesothelioma: an overview

Several molecular defects have been described in malignant mesothelioma cells. These include mutations affecting the *CDKN2A* and *BAP1* gene, aberrant activation of the Wnt

pathway and up-regulation of several receptors including epidermal growth factor receptors (EGFR) [5], AXL, insulin-like growth factor-1 receptor, vascular endothelial growth factor receptor (VEGFR)-2, RON and Met [6].

Physiologic function of the hepatocyte growth factor/Met axis

The Met receptor tyrosine kinase (RTK) is activated upon binding by a single ligand species, named hepatocyte growth factor (HGF). HGF is principally secreted by a variety of mesenchymal cell types, including fibroblasts, vascular smooth muscle cells and other stromal cells, whereas Met is primarily expressed on the surface of epithelial cells. By this arrangement, the HGF/Met axis establishes a mesenchymal–epithelial communication pathway that regulates a number of physiologic processes, including embryogenesis, organ development, wound healing, angiogenesis and tissue homoeostasis and regeneration [7]. In keeping with these fundamental activities, mice that are null for either *MET* or HGF are embryonically lethal [8–10]. Several functional consequences ensue after the binding of HGF to Met, including enhanced cell survival, proliferation, cell movement and branching morphogenesis.

The HGF/Met axis and mesothelioma

Although activity of the HGF/Met system is required for development, expression of these binding partners is generally found at low levels in healthy adult tissues. By contrast, dysregulation of the HGF/Met system (Figure 1) is prevalent in many lung and other cancers, including malignant mesothelioma, and may result from mutation, gene amplification or protein over-expression [6].

HGF expression in mesothelioma

Harvey and colleagues were first to investigate the expression of Met and HGF in malignant pleural mesothelioma (MPM) [11]. Using immunohistochemistry, they demonstrated that nine of nine MPM tumours (representing all three histological subtypes) were reactive for HGF. Eight of the tumours were graded as intensely positive, with uniform and diffuse cytoplasmic staining evident in tumour cells together with frequent stromal reactivity. In agreement with this finding, HGF was also detected in four of four tested MPM-associated pleural effusions [12].

Tolnay et al. subsequently assessed a larger number of MPMs and found that 33 of 39 stained positively with anti-HGF antibody. Once again, reactivity was evident in both in tumour cells and accompanying stroma [13]. Although virtually all epithelioid and biphasic MPM were positive, the authors noted that biphasic tumours exhibited stronger staining in the epithelioid compared with the sarcomatoid portion. Furthermore, only one in eight of the sarcomatoid tumours tested positive for HGF. Normal mesothelial cells were negative, a finding that contrasts with an earlier report of weak HGF expression in four of eight cases [11]. Expression of HGF (but not Met) was correlated with tumour microvessel density, in keeping with the known role of HGF in promoting angiogenesis [13].

In keeping with these findings, serum levels of HGF (and EGF) are elevated in patients with mesothelioma, compared with healthy individuals of a similar age [14]. However, autocrine release of HGF is uncommon in immortalised MPM cell lines [15,16], having primarily been described in cell types with a sarcomatoid or mixed phenotype [17]. By analogy with other tumour types, this raises the possibility that stroma is also an important source of HGF production in mesothelioma. In keeping with this, Li et al. [18] have presented evidence that mesothelioma cells release platelet-derived growth factor and fibroblast growth factor, which in turn promote an influx of activated HGF-secreting fibroblasts into the tumour microenvironment. This suggests that a paracrine relationship between stroma and tumour cells may also serve to reinforce disease progression in mesothelioma.

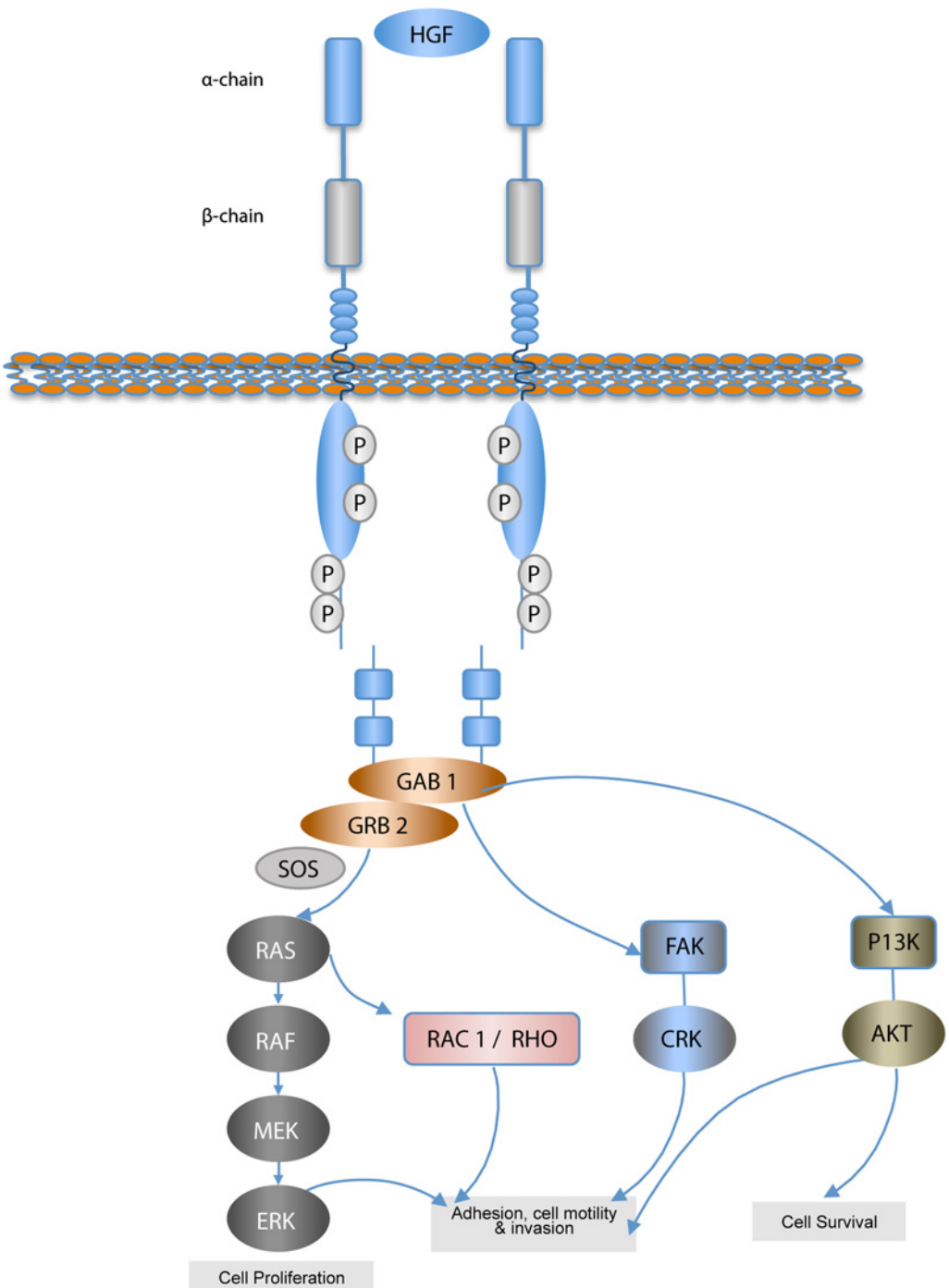
Met expression in mesothelioma

Using immunohistochemistry, Harvey et al. also demonstrated strong expression of the Met receptor in MPM tumour cells and associated stroma [11]. Once again, staining was diffuse and mainly cytoplasmic, with some membranous reactivity also apparent. Furthermore, in all six MPM-associated pleural effusions where tumour cells were identified, Met expression was detected [19]. These findings were subsequently extended in four larger series, which respectively demonstrated that 74% ($n = 39$) [13], 80% ($n = 35$) [20], 82% ($n = 66$) [14] and 76% ($n = 157$) [21] of MPM tumours tested positive for Met expression. Similar to HGF staining, expression was most common in epithelioid tumours, least frequent in sarcomatoid variants, whereas in biphasic MPMs, reactivity was most apparent in the epithelioid component [13]. Fluorescence *in situ* hybridisation confirmed a direct correlation between Met expression in tumours at the mRNA and protein levels [13]. In the majority of tumours tested, Met phosphorylation was detected [20,21]. In mesotheliomas with high-level expression, Met was predominantly located on the plasma membrane, whereas cytoplasmic staining was predominantly seen in tumours where expression was lower [21]. Intriguingly however, high plasma membrane expression of Met was associated with improved outcome, although the authors note that this finding requires independent validation [21]. By contrast, normal mesothelial cells have generally been found to lack Met expression at the protein [13] or RNA level [19]. Nonetheless, polarised expression along the apical surface was demonstrated in normal mesothelium in one study [11].

In keeping with these findings, studies of both human and murine mesothelioma cell lines have confirmed that the

Figure 1 | **The HGF/Met signalling pathway**

The main signalling pathways activated by the HGF/Met axis. HGF binding to Met stimulates receptor dimerisation activating different signalling pathways. Upon activation, Met kinase domain mediates signalling via adaptor proteins and other signalling proteins leading to (i) proliferation through MAPK activation via Gab1–Grb2 resulting in Ras activation; (ii) cell survival via the PI3K and Gab1 mediated activation of AKT resulting in an anti-apoptotic response; (iii) adhesion and cell motility mediated via Ras activation and direct interaction with the PI3K–FAK pathway which contributes to the invasive phenotype.



majority of those tested express the Met receptor [19,20]. Comparison with an immortalised cell line derived from healthy mesothelium indicates that *MET* is transcriptionally up-regulated in 75 % of cases [20].

MET mutation in mesothelioma

A number of *MET* mutations have been identified in primary MPM and derived cell lines [14]. Overall however, it has been estimated that somatic mutation of *MET* is only detectable in approximately 3 % of such tumours [16]. Similarly, no activating *MET* mutations were identified in an assessment of 30 mesothelioma cell lines [15,20]. A polymorphism (T1010I) that affects the juxtamembrane regulatory domain of Met has been identified in a small number of cases of MPM [16] and in derived cell lines [14]. Although this polymorphism has the capacity to transform an IL-3-dependent cell line, it remains uncertain whether this finding has clinical relevance in mesothelioma [16].

Factors that dysregulate the HGF/Met axis in mesothelioma

At least five factors have been identified that have the capacity to dysregulate expression of HGF and/or Met in mesothelial or other cell types and thus may have relevance to mesothelioma pathogenesis.

Exposure of murine mesothelial cells to crocidolite (the most pathogenic form of asbestos) promotes up-regulation of the Fra-1 (fos-related antigen-1) proto-oncogene. This in turn leads to AP-1-dependent Met up-regulation [22]. Furthermore, increasing evidence from a number of tumour systems suggests that hypoxia can drive the transcriptional up-regulation of Met expression [23].

Related to this, Adamson and Bakowska [24] performed an *in vivo* study in which rats were exposed to crocidolite asbestos, delivered by intratracheal instillation. As a result, a proliferative burst of bronchoalveolar epithelium and pleural mesothelial cells ensued. To dissect mechanisms, they demonstrated that HGF and keratinocyte growth factor levels increased in bronchoalveolar and pleural lavage fluid over the following days. Evidence that both cytokines contributed to mesothelial cell proliferation was supported by antibody blocking studies.

Genetic factors may conspire to up-regulate the HGF/Met axis. Studies using a murine model of asbestos-induced mesothelioma have indicated that haplo-insufficiency for both *CDKN2A* and *NF2* enhances tumour aggressiveness, associated with up-regulated expression and activation of Met and expression of stem cell-associated attributes [25]. In addition, loss of function p53 mutation favours Met up-regulation, perhaps via dysregulated microRNA expression [26].

Finally, the SV40 tumour virus has been linked in a number of studies to mesothelioma development, although this remains controversial [27]. Notably however, in mesothelioma cell lines that express the large T antigen (an SV40-derived oncoprotein), Met is constitutively phosphorylated as a result of the establishment of an HGF autocrine loop [28].

Consequences of aberrant activation of the HGF/Met axis in mesothelioma

Addition of HGF (either recombinant or derived from tumour samples) to mesothelioma-derived cell lines results in increased phosphorylation of Akt [29], Erk1/2 and Met itself [14]. As a result, these cells exhibit an increase in non-directional motility, chemotactic migration, altered morphology, cell division and invasiveness *in vitro* [19,30].

In vivo studies using cell lines have also shed light on possible effects of activation of the Met/HGF axis in malignant mesothelioma. When an autocrine Met/HGF loop is established in NIH3T3 fibroblasts, these cells acquire tumorigenic capacity when inoculated in nude mice [31]. Tumours aberrantly express a number of epithelial markers, suggesting that Met signalling can promote epithelial transdifferentiation of these mesenchymal cell types [32]. This finding is noteworthy since mesotheliomas commonly display both epithelial and mesenchymal/sarcomatoid features and molecular markers [33].

Therapeutic targeting of the HGF/Met axis in mesothelioma – a viable option?

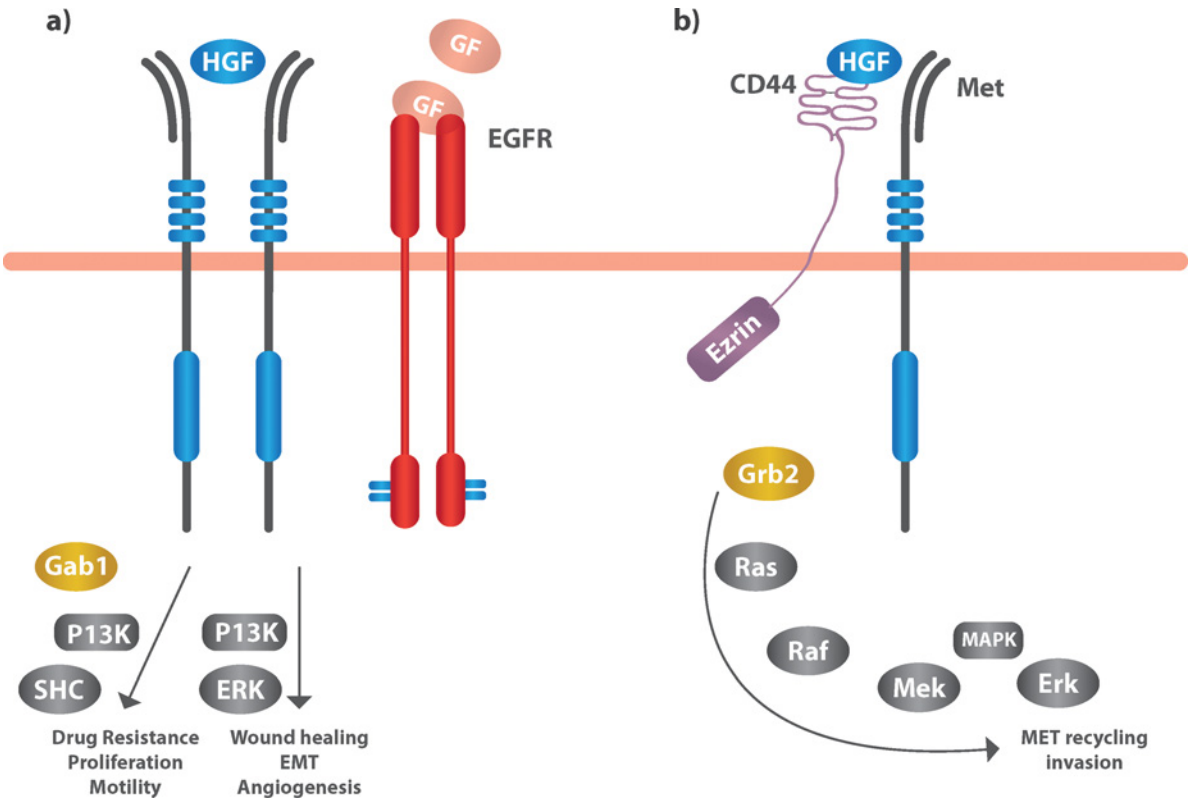
Several clinical trials are currently evaluating therapeutic agents directed against the HGF/Met axis, in solid and some haematologic malignancies. A list of current studies can be found in [34].

Targeted therapies directed against Met may be considered in three categories [35]. First, decoy ligands, such as truncated splice variants of HGF may be used to competitively inhibit ligand binding to Met. One such example is NK4, in which only the N-terminal hairpin and four Kringle domains within HGF are present. Although NK4 can bind to Met, it does not promote receptor phosphorylation and this acts as a competitive inhibitor. However, if HGF β-chain is subsequently added, Met receptor phosphorylation and function is then reconstituted [36]. Pre-clinical studies indicate that NK4 can inhibit mesothelioma growth, both *in vitro* and *in vivo* [37]. Intriguingly however, this action is partly independent of Met inhibition and may reflect additional direct anti-tumour effects and its anti-angiogenic action. The latter is believed to result not only from Met inhibition but also from the ability of NK4 to bind to the heparan sulfate glycoprotein, perlecan. As a result, perlecan on the cell surface of integrin-expressing endothelial cells can no longer engage with fibronectin, following stimulation with other pro-angiogenic factors [38].

The second category comprises monoclonal antibodies directed against either HGF (e.g. rilotumumab, ficlatuzumab, TAK701) or Met (e.g. onartuzumab, LY2875358) [34]. However, none of these agents are presently being evaluated in mesothelioma.

Figure 2 | **Cross-talk between Met and cell surface proteins**

(**a**) The interaction between Met and EGFR results in the activation of the PI3K signalling pathway leading to increased drug resistance, proliferation and a greater angiogenic phenotype. (**b**) HGF-dependent Met and CD44 interaction leads to members of the MAPK signalling pathway such as MEK and Erk being activated, resulting in an invasive phenotype.



Third, several small molecule Met kinase inhibitors are currently undergoing evaluation in patients with advanced Met-expressing malignancy [34]. Agents include AMG 208, AMG 337, BMS-777607, EMD 1204831, EMD 1214063, INCB028060, LY2801653, MK8033, MSC2156119J, PF-04217903 and volitinib. Also under study are the Met/VEGFR-2 dual kinase inhibitors, golvatinib and foretinib, the dual ALK/Met inhibitor, crizotinib and the multikinase inhibitors: amuvatinib, MGCD265, cabozantinib and MK2461 (preferential inhibitor of Met). Preclinical studies have demonstrated that Met kinase inhibitors can suppress the growth of some mesothelioma cell lines, most notably those containing the T1010I polymorphism [14] or in which a HGF/Met autocrine loop is demonstrable [15]. Although both situations occur in a minority of mesotheliomas, the *in vivo* situation is undoubtedly more complex since paracrine stimulation by HGF appears to occur commonly [18]. A multicentre Phase 2 clinical trial evaluating a selective Met inhibitor, tivantinib, is currently ongoing in patients with mesothelioma who have failed prior therapies (NCT01861301, clinicaltrials.gov, search performed October 26th, 2015). However, results will need to be interpreted in the light of increasing evidence that this and other Met inhibitors exert significant off target effects involving other kinases [39].

Caught in the crossfire: interactions between Met and other signalling networks

Increasing evidence indicates that aberrantly activated receptor/ligand systems do not operate in isolation, but instead engage in cross-talk with other pathways in healthy and transformed cells. Many examples of such 'cross-talk' have been described in relation to the HGF/c-Met axis and may afford opportunities for the emergence of tumour cell resistance to the targeted inhibition of this pathway in isolation [26].

Previous studies have shown complex interactions between HGF/Met with other membrane RTKs such as Semaphorin-4D/Plexin B1 [40] and SDF1/CXCR4 [41], suggesting the importance of cross-talk between membrane receptors of various types.

Cross-talk between Met and other receptor tyrosine kinases

The Met receptor engages in cross-talk with several other RTKs, many of which are co-expressed in malignant mesothelioma. The best characterised of these interactions occurs between Met and EGFR (Figure 2a). Amplification of *MET* is a well-recognised mechanism of mediating resistance

of EGFR-mutant lung cancer cells to EGFR kinase inhibitors [42]. Stimulation of MPM cells with HGF causes the phosphorylation of EGFR whereas knockdown of *MET* or inhibition of Met kinase activity can lead to reduced EGFR phosphorylation [16]. The converse relationship also applies in that EGF stimulation of MPM cells promotes the enhanced phosphorylation of Met. In mesothelioma, the EGFR is up-regulated in the majority of tumours and is phosphorylated in virtually all cases [43]. Nonetheless, EGFR inhibitors have proven ineffective to date when used in isolation to treat patients with mesothelioma [44]. Pre-clinical studies using a panel of mesothelioma cell lines have demonstrated that combined inhibition of both Met and EGF receptor achieved greater suppression of cell growth, migration and invasion, compared with selective targeting of either receptor alone [20]. Furthermore, the AKT inhibitor perifosine also reduced the ligand-induced phosphorylation of EGFR and Met receptors in mesothelioma cell lines, accompanied by inhibition of proliferation and enhanced sensitivity to platinum agents [45]. These data raise the possibility that Met signalling can buffer against the inhibitory effects of EGFR blockade in mesothelioma and raise the prospect that combined inhibition of both pathways may warrant clinical evaluation.

Studies of other model systems provide evidence of cross-talk between Met and other RTKs. The HGF/Met axis is closely related to a second ligand receptor pair, comprising macrophage stimulating protein (MSP) and the Ron RTK [7]. In gastric carcinoma models, heterodimerisation and cross-phosphorylation of the Met and RON receptors has been demonstrated upon binding of either HGF or MSP [46]. Such a mechanism may also operate in mesothelioma since previous preliminary data suggest that both MSP and RON are commonly expressed in this tumour [47]. Furthermore, the AXL receptor is also commonly expressed in mesothelioma [48] and signals co-operatively and in a bidirectional manner with HGF/Met in some models [49]. There is also evidence that other RTKs such as IGF-1R, RET [50] and VEGF receptor [51] can elicit the ligand-independent transactivation of the Met receptor. Co-operative signalling between Met and ErbB2/ErbB3 has also been described [26]. Together, these findings place Met as an effector of signalling by several other receptor systems and warrants further investigation of the intermediates that relay such signals and which may provide important targets for therapeutic exploitation.

Cross-talk between Met and other receptor types

Emerging evidence also suggests that cross-talk between Met and other (non-tyrosine kinase) receptor types also occurs (Figure 2b). In several cellular systems, CD44 receptor isoforms containing the variant 6 exon (CD44v6) are required in order that HGF can elicit Met activation, through ternary complex formation [52]. In keeping with this, peptides derived from the 42 amino acid-containing variant exon 6 can inhibit the binding of HGF to Met and thereby abrogate function [53]. The CD44 variant exon 3 contains a heparin sulfate-binding site, enabling isoforms that contain this exon to bind HGF, perhaps facilitating the more efficient capture and presentation of this ligand to Met [26]. HGF has also been reported to promote the association of Met with CD44 containing variant exon 10, leading to efficient phosphorylation of Met and recruitment into caveolin-enriched microdomains [54]. Although CD44 is commonly up-regulated on mesotheliomas [55], there has been relatively little study of which CD44 variants are expressed in this disease. One study has suggested that CD44v6 may be under-represented in mesothelioma compared with other lung tumours [56]. To add complexity, one previous study in CD44-deficient mice suggests that ICAM-1 may alternatively be recruited to provide this co-receptor function [57] and small studies in mesothelioma suggest that ICAM-1 is very commonly and highly expressed in this tumour [58].

Transactivation of Met (and several other RTK) has also been reported upon stimulation of several G protein coupled receptors [26,59]. Additionally, ligand-independent Met activation has been described following integrin engagement [60]. In keeping with this, Met can physically associate with a number of integrins [26]. Furthermore, full Met functioning in some models requires complex formation with the $\alpha 3\beta 1$ [61] or $\alpha 6\beta 4$ integrin [62].

Another example of this type of cross-talk is the ability of Met to associate with Semaphorin receptors, namely Plexins and Neuropilins [63]. For example, Sema4D (a Plexin B1 ligand) increases the phosphorylation of both its receptor and Met leading to enhanced tumour invasiveness [64], although the importance of this interaction in mesothelioma is unclear.

Conclusions

Dysregulation of the HGF/Met axis is prevalent in malignant mesothelioma. Furthermore, emerging evidence suggests that cooperative signalling with other ligand–receptor systems may contribute to disease pathogenesis. Consequently, combinatorial targeting approaches may prove more effective in this situation. Greater understanding of the integrated signalling network within which Met operates in mesothelioma will provide opportunities for the development of novel targeted therapeutic approaches for this devastating cancer.

Acknowledgements

Mesothelioma research in the author's laboratory is supported by the British Lung Foundation, by the Experimental Cancer Medicine Centre at King's College London and by the National Institute for Health Research (NIHR) Biomedical Research Centre (BRC) based at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Funding

Research undertaken by the authors related to this review has been supported by a British Lung Foundation PhD studentship, the

Experimental Cancer Medicine Centre at King's College London and by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

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Received 7 January 2016
doi:10.1042/BST20150252