HGF/Met Signaling in Head and Neck Cancer: Impact on the Tumor Microenvironment

Stefan Hartmann¹,², Neil E. Bhola¹, and Jennifer R. Grandis¹

Abstract

Studies to date have revealed several major molecular alterations that contribute to head and neck squamous cell carcinoma (HNSCC) initiation, progression, metastatic spread, and therapeutic failure. The EGFR is the only FDA-approved therapeutic target, yet responses to cetuximab have been limited. Activation and cross-talk of cellular receptors and consequent activation of different signaling pathways contribute to limited activity of blockade of a single pathway. The hepatocyte growth factor (HGF) receptor, Met, has been implicated in HNSCC tumorigenesis and EGFR inhibitor resistance. HGF, the sole ligand of Met, is overexpressed in the tumor microenvironment. The role of HGF/Met signaling in proliferation, metastasis, and angiogenesis has been investigated in HNSCC, leading to clinical trials with various Met inhibitors and HGF antibodies. However, the role of the HGF/Met signaling axis in mediating the tumor microenvironment has been relatively understudied in HNSCC. In this review, we discuss the functional roles of Met and HGF in HNSCC with a focus on the tumor microenvironment and the immune system.

Introduction

The annual incidence of head and neck cancer (HNC) worldwide is about 650,000 cases (1). In 2015, almost 60,000 patients were diagnosed with a malignancy of the oral cavity, pharynx or larynx in the United States (2). Although 95% of HNC are squamous cell carcinomas (HNSCC), previous and ongoing genetic profiling underscores the distinct heterogeneity of this entity (3, 4). However, one common observation in up to 90% of the HNSCCs is the overexpression of EGFR (5).

Major risk factors for the development of HNSCC include tobacco use, excessive alcohol consumption, and human papillomavirus (HPV) infection. Impaired oral hygiene and genetic alterations resulting in susceptibility to malignancies such as Fanconi anemia have also been implicated as risk factors. Depending on site and tumor stage, therapeutic options include surgery, irradiation, and chemotherapy. Cetuximab, an FDA-approved mAb targeting EGFR, is the only targeted therapy approved for this disease.

HGF/Met Pathway

The mesenchymal epithelial transition (Met) factor receptor is a receptor tyrosine kinase (RTK) that is encoded by the MET proto-oncogene (9). Briefly, the Met receptor consists of a 45 kDa extracellular α-chain, linked to a 145-kDa transmembrane β-chain via disulphide bonds (10). Upon binding to its ligand HGF, two Met receptors dimerize leading to autophosphorylation of three tyrosine residues (Y1230, Y1234, Y1235; refs. 11, 12; Fig. 1). Following this initial phosphorylation cascade, phosphorylation of two other tyrosine residues (Y1349, Y1356) occurs and these residues serve as docking sites for downstream signaling molecules that mediate Ras/Raf, PI3K/Akt/mTOR, and/or STAT3 pathways (13–15). Met activation has been extensively shown to drive proliferation, migration, invasion, and angiogenesis in HNSCC and other tumor types (16) and HGF/Met activation is a known mechanism of resistance to anti-EGFR therapy (17).

Targeting approaches to the HGF/Met signaling axis is mostly comprised of mAbs (directed against Met or HGF), tyrosine kinase inhibitors (TKI), and/or a NK4 decoy, which is a HGF antagonist (18). Most preclinical studies and clinical trials have focused on the mAbs (e.g., fلافatuzumab, rilotumumab, onartuzumab) or TKIs (e.g., foretinib, crizotinib, tivantinib), leading to phase III studies for tivantinib and crizotinib in lung cancer (NCT01377376 and NCT01662869, respectively) or rilotumumab in gastric cancer (NCT01662869). Importantly, only crizotinib and caboziotanib have received FDA approval for lung adenocarcinoma (19, 20) and RET-positive medullary thyroid carcinoma (21), respectively. Moreover, caboziotanib has shown activity in renal cell carcinoma (22) and was recently FDA approved for this disease.

HGF/Met in HNSCC

Genomic and proteomic data

More than 20% of HNSCC harbor either a copy number gain or amplification of MET (23, 24) and more than 80% show Met...
protein overexpression (ref. 25; Fig. 2). The Met ligand, HGF, which is secreted by cells in the surrounding tumor microenvironment in a paracrine manner (26) is overexpressed in about 50% of head and neck squamous cell carcinoma (HNSCC)-associated stroma (8, 25).

The HNSCC TCGA data suggest that the mutation frequency of Met is less than 1% in primary tumors, (23, 24). Similarly, MET gene alterations are rare and not predictive of response to therapy. Interestingly, in a cohort of 143 HNSCC patients, one group found six cases (4%) with a MET gene mutation (27). In contrast, a frequency of 11% for the Y1253D mutation in another cohort of 138 oropharyngeal squamous cell carcinomas was reported (28). However, there is some evidence that the constitutively active Y1253D Met mutation may undergo clonal selection during tumor spreading and metastasis. This may explain a crucial role for activating Met mutations, although their frequency in primary tumors seems to be very low (29). Noteworthy, the mutation frequency, in particular of small lesions (e.g., exon 14 skipping), might be underestimated due to technical difficulties in detection (30). Exon 14 skipping itself is associated with MET amplification and Met overexpression (31).

### Proliferation

Malignant cells are defined by characteristics that can differentiate them from normal cells (32). Ongoing proliferative signaling, in particular upregulation of receptors, ligands, or circumvention by feedback mechanisms, is a major tumor cell characteristic (32). Tumor-associated fibroblasts (TAF), not HNSCC cells per se, are the major source of HGF in the tumor microenvironment (26). Strikingly, tumor cell-conditioned media engages the TAFs to produce and secrete even higher amounts of HGF than being cultured in control media, showing a mutual interaction between both compartments (33). Furthermore, epithelial cancers coexpress Met and matriptase, a cell surface-anchored protease, that activates the HGF precursor, pro-HGF (34). HGF-mediated activation of Met results in enhanced cell proliferation and tumor growth in HNSCC (26, 35). In combination, these capabilities result in a sustainable production of growth factors to nuture a proliferative tumor niche.
**Invasion, migration, and metastasis**

The vast majority of research on Met in HNSCC has been focused on invasion, migration, and metastasis. HGF-induced migration and invasion of HNSCC was mitigated by ficlatuzumab (36), a HGF-directed antibody that is currently under clinical investigation (NCT02277197). In line with these findings, Tao and colleagues reported impaired cancer cell motility, decreased lymph node metastasis and prolonged overall survival following Met knockdown in an *in vivo* model of HNSCC (35). Moreover, Met expression is elevated in primary tumors with advanced

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**Table 1. Results from preclinical studies with agents targeting HGF/Met in head and neck cancer**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Condition</th>
<th>Main findings</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Crizotinib</td>
<td><em>In vitro</em></td>
<td>Crizotinib enhanced radiosensitivity in one cell line but failed to enhance radiosensitivity in cell line xenografts.</td>
<td>(84)</td>
</tr>
<tr>
<td>Crizotinib</td>
<td><em>In vitro</em></td>
<td>Crizotinib in combination with erlotinib significantly decreases cell proliferation and invasion <em>in vitro</em>. <em>In vivo</em> combination treatment resulted in higher rate of apoptotic cells, lower rate of proliferating cells, and a nearly significant effect on tumor volume as compared to single treatment (<em>P</em> = 0.0525).</td>
<td>(85)</td>
</tr>
<tr>
<td>Crizotinib</td>
<td><em>In vitro</em></td>
<td>Crizotinib inhibits Met activation and wound closure <em>in vitro</em>. <em>In vivo</em> cell line xenograft models crizotinib enhances apoposis and inhibited tumor growth.</td>
<td>(25)</td>
</tr>
<tr>
<td>Crizotinib</td>
<td><em>In vitro</em></td>
<td>Crizotinib impairs sphere formation <em>in vitro</em>. Crizotinib enhances growth inhibition of docetaxel and cisplatin in patient-derived xenograft (PDX) models. Crizotinib inhibits the CSC-like population and impaired metastatic spread in PDX models.</td>
<td>(86)</td>
</tr>
<tr>
<td>Ficlatuzumab</td>
<td><em>In vitro</em></td>
<td>Ficlatuzumab inhibits TAF-facilitated cell proliferation, migration and invasion. Ficlatuzumab inhibits Met activation in HGF-treated cells.</td>
<td>(36)</td>
</tr>
<tr>
<td>PF04217903</td>
<td><em>In vitro</em></td>
<td>PF04217903 enhances erlotinib sensitivity in erlotinib-resistant cell lines. In cell line xenograft models, PF04217903 in combination with erlotinib decreases tumor growth and proliferation and increases apoptosis.</td>
<td>(87)</td>
</tr>
<tr>
<td>SU11274</td>
<td><em>In vitro</em></td>
<td>SU11274 decreases cell viability, motility, and migration. SU11274 synergies with erlotinib and cisplatin in decreasing cell viability.</td>
<td>(25)</td>
</tr>
<tr>
<td>SU11274</td>
<td><em>In vitro</em></td>
<td>SU11274 inhibits Met activation, wound closure, and IL8 secretion.</td>
<td>(26)</td>
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<tr>
<td>SU11274</td>
<td><em>In vitro</em></td>
<td>SU11274 decreases sphere-forming capacity and stem cell marker expression (Oct-4, SOX2).</td>
<td>(40)</td>
</tr>
<tr>
<td>Tivantinib</td>
<td><em>In vitro</em></td>
<td>Tivantinib suppresses cell proliferation and colony formation and induces cell-cycle arrest and caspase-dependent apoptosis.</td>
<td>(88)</td>
</tr>
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</table>

Abbreviation: CSC, cancer stem cell.

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**Figure 2.**

Clinical significance of the HGF/Met pathway in HNSCC. A, the Kaplan–Meier curve for overall survival shows distinct differences between the group without alterations (gain or amplification of gene copy number) in HGF or MET and the group with alterations of HGF and/or MET (*P* = 0.0118). The overall survival after 60 months is 49.8% in patients without HGF/MET alterations and 38.2% for patients with alterations in HGF and/or MET. B, Alterations in MET gene copy number occur in approximately 23% (5/530 samples with an amplification; 114/530 samples with a copy number gain). The graph is cropped and does not show the complete number of unaltered cases (colored in gray).
lymph node metastasis (N2/N3) compared with early-stage disease (N0/N1), suggesting its role as a metastatic driver in HNSCC (37). Under normal conditions, loss of cell–cell contact results in cell death, a process known as anoikis. HGF was reported to inhibit anoikis in HNSCC cells via Akt and ERK signaling (38). The capability to circumvent anoikis has been described as an indicator of invasive/metastatic capacity and underscores the importance of HGF/Met signaling.

Cancer stem cells
Met is thought to contribute to a cancer stem cell (CSC)-like phenotype in HNSCC. CSCs are a population of cells within a tumor that possess the ability to self-renew, evade drug action, and reconstitute a heterogenous tumor (39). HGF treatment enhances sphere-forming capacity and also increases the expression of stem cell markers OCT4, SOX2, and CD44 (40). In HNSCC cells, SOX2 expression contributes to increased proliferation, self-renewal, invasive capacity, and cisplatin resistance. Furthermore, SOX2 expression is correlated with tumor recurrence and decreased survival in HNSCC patients (41). Sun and colleagues reported that cisplatin treatment of HNSCC cells upregulates Met expression in vivo as compared with untreated controls. Interestingly, these cells showed enhanced secondary tumor growth when injected into mice in a limiting dilution assay. Furthermore, HNSCC CSCs with higher Met levels show an enhanced metastatic ability as compared with low expressing Met CSCs (42). Moreover, differences in Met mRNA levels were shown when comparing radiosensitive and radioresistant HNSCC cells (43). Following irradiation, Met expression was diminished in the radiosensitive cell lines; however, Met expression was elevated in the radioresistant HNSCC models. These cumulative findings indicate that Met plays a critical role in therapeutic resistance by promoting a cancer stem cell–like phenotype.

HGF/Met targeting strategies

Preclinical studies. On the basis of the understanding of HGF/Met signaling and its role in carcinogenesis, metastasis and resistance to several therapeutic approaches, a large number of agents have been developed to target this signaling axis. In general, three different groups of agents were described in the past: (i) mAbs, either targeting the ligand HGF or the receptor Met, (ii) tyrosine kinase inhibitors, targeting the tyrosine kinase domain of Met (and mostly also the TK domain of other RTKs), (iii) a truncated, soluble Met receptor serving as decoy for HGF (44) and the competitive HGF antagonist NK4 (18). Table 1 provides an overview on agents used in preclinical in vitro and in vivo HNSCC models.

Clinical studies. The number of ongoing clinical studies targeting the HGF/Met axis in head and neck cancer is, compared with other solid tumors, relatively small. As of June 2016, only five phase I or II trials were registered at ClinicalTrials.gov (Table 2). In two phase I studies, ficlatuzumab is the investigational drug, either in combination with cetuximab (NCT02277197) or with cisplatin/IMRT (NCT02277184). Interestingly, in the study with ficlatuzumab plus cisplatin/IMRT, the patients enrolled will have an intermediate or high risk, locally advanced HNSCC but no recurrent or metastatic disease. The study investigating ficlatuzumab plus cisplatin/IMRT is suspended.

Two other studies investigate capmatinib plus cetuximab (phase Ib; NCT02205398) or tivantinib plus cetuximab versus cetuximab alone (phase II; NCT01696955). However, for both studies no preliminary results are available yet. The only completed clinical study investigated foretinib in single-agent use in recurrent/metastatic patients. The study was initially designed as two-step study, enrolling additional patients (n = 27) after observing at least one response (partial response or complete response) within the first group of 14 patients. However, the best outcome was stable disease in 7 of 14 patients. Three patients showed disease progression, one patient was unable to evaluate and three patients had no on-treatment scan. As the goal for entering the second step was not achieved, the study was terminated at this point (45).

The Tumor-Extrinsic and Tumor-Intrinsic Role of the HGF/Met Pathway

HGF and the tumor microenvironment
The tumor microenvironment (TME) is a complex tissue structure that consists of fibroblasts, blood vessels, several immune cells, and the extracellular matrix (ECM; ref. 46). Importantly, the
TME does not only surround the tumor cells, it actively contributes to tumor development and progression (47), drug resistance (48), and metastasis (49).

Tumor cell–stimulating HGF is secreted in a paracrine manner by TME-localized fibroblasts and not by the tumor cells themselves (26). Fibroblasts cocultured with tumor cells secrete higher amounts of HGF as compared with fibroblasts cultured in the absence of tumor cells, showing a mutual interaction between the tumor and its surrounding tissue (ref. 26; Fig. 2). Several studies concluded that Met amplification is a predictor for efficacy of Met-targeted therapies (50). However, most of these studies were performed in the absence of HGF, excluding the fact that HGF is generally present in the tumor and its microenvironment. In this context, Pennacchietti and colleagues showed that HGF inhibited the antitumor effects of Met-directed TKI and mAbs even in highly sensitive MET-amplified tumor cells (51). The HGF targeting antibody fclutzumab sensitized MET-amplified cells to Met-directed TKIs and mAbs (51).

Although MET amplification is a rare event in HNSCC, targeting HGF is a rational approach because HGF overexpression in the TME is found in about 50% of HNSCC patient specimens (8, 25). Furthermore, HNSCC patients display increased HGF serum levels compared with healthy individuals (52) and HGF levels in the primary tumor positively correlate with metastasis (52). Preclinical results demonstrating the ability of fclutzumab to mitigate the effects of tumor-associated fibroblasts on proliferation, migration, and invasion were recently published (36).
TME-derived HGF was shown to enhance radioreistance and chemoresistance in several cancer entities (53, 54). Also in HNSCC, there is some evidence that HGF/Met signaling might be associated to Bcl-xl expression (55) and radioreistance (56). Chronic HGF stimulation, which is present in the TME, augments glucose influx and membrane expression of the glucose transporters GLUT-1 and GLUT-4 in a myocyte model (ref. 57; Fig. 3). The enhanced influx of glucose by malignant cells is necessary to satisfy their energy requirements (aerobic glycolysis, "Warburg effect"; ref. 58). Similarly, Kaplan and colleagues reported enhanced glucose consumption and lactate production after HGF stimulation in a breast cancer model (59). In a NSCLC model, inhibition of Met with PHA-665752 resulted in downregulation of hexokinase 2 (HK2), which is important for the initiation of glycolysis (ref. 60; Fig. 3). Furthermore, Met inhibition significantly decreased phosphorylated pyruvate kinase isozyme 2 (p-PKM2), a further key factor in maintaining the Warburg effect in cancer cells (ref. 58; Fig. 3). These studies indicate that HGF stimulation may drive resistance in a glycolysis-dependent manner.

As a result of fuelling glycolytic pathways, higher levels of lactate are produced by the tumor cells, which is secreted in the TME by monocarboxylate transporters (MCT). The HNSCC TCGA data shows a significant co-occurrence of elevated HGF and MCT-1/4 mRNA levels ($P = 0.032$ and $P < 0.001$, respectively; refs. 23, 24). Lactate is not only a glycolytic waste product as it leads to lactate production after HGF stimulation in a breast cancer model (59). In a murine model, deletion of Met led to enhanced HNSCC tumor cell migration and inhibits monocyte activation and migration (61), underscoring the connection between HGF, altered energy metabolism, the tumor microenvironment and the immune system.

Met and antitumor immune response

The interactions between the tumor and the immune system in the tumor microenvironment is increasingly appreciated as a critical pathway amenable to therapeutic manipulation. Innate and adaptive immunity play important roles in suppressing or promoting tumorigenesis. For example, M1-polarized macrophages mediate tumor cell death while the M2 macrophages promote tumor growth (62, 63). Stimulation of macrophages with HGF results in differentiation of M1 macrophages to M2 subtypes (64), underscoring the functional relevance of HGF/Met axis in the antitumor immune response.

There is evidence that HGF/Met signaling results in higher lactate secretion by cancer cells via upregulation of glycolysis (65). Lactate was shown to potently suppress the proliferation and activity of human CTLs (66). CTL inactivation negatively correlates with recurrence-free and overall survival in HNSCC (67). Of note, mesenchymal stem cells (MSC) produce HGF, which activates and expands the myeloid-derived suppressor cells (MDSC) in a STAT3-dependent manner (68). Activated MDSCs suppress the expansion of CTLs and further expand the immunosuppressive T regulator cell populations (69).

Depending on their role (antitumor functions or protumorigenic role), neutrophils can be classified as N1 or N2 (70), implicating that their role in cancer is ambivalent. The role of HGF/Met signaling in neutrophils is unclear. Neutrophils contain pro-HGF that can be cleaved, activated, and released upon activation (71). For instance, in bronchoalveolar carcinoma, neutrophil infiltration (and HGF release) is considered a negative prognostic marker (72). However, HGF/Met signaling in neutrophils can also have antitumor effects. As recently shown in a murine model, deletion of Met led to enhanced growth and metastasis of transplanted or endogenously induced tumors by reduced neutrophil infiltration (73).

The activation of T cells is also modulated by dendritic cells (DC). In a model of experimental autoimmune encephalitis, HGF is a potent immunomodulatory factor that substantially inhibits antigen-presenting function of DC, induces expansion of CD25+ Foxp3+ regulatory T cells, and increases IL10 production (74). IL10 impairs DC differentiation from stem cells (75) and enhances DC apoptosis (76). In cancer models, this effect of IL10 has been shown to protect tumor cells from CTLs (77). Importantly, the HGF-associated immunosuppressive effects were fully reversed after treatment with a Met antibody. In multiple myeloma, high levels of serum HGF correlate with disease burden and immune system impairment by upregulation of indoleamine 2,3-dioxygenase 1 (IDO1; ref. 78). Importantly, multiple myeloma is a malignancy where programmed cell death protein 1 (PD-1) and its ligand (PD-L1), is critical for immune evasion and tumor progression (79).

Nowadays, the most actively investigated immunotherapeutic target is PD-1 and its ligand PD-L1, which serve as immune checkpoints. PD-1 is predominantly expressed on T cells, and upon binding to PD-L1, T-cell receptor (TCR)-mediated activation is inhibited (80). A recent report demonstrated that HGF-stimulated renal cancer cells displayed PD-L1 upregulation and colocalization with Met (ref. 55; Fig. 3). Notably, HGF-mediated upregulation of PD-L1 was dependent on the PI3K pathway which is frequently mutated and activated in HNSCC (81). In addition, Malm and colleagues reported that 80% of HNSCC specimens express PD-L1 (82). Currently, a phase III trial which investigates nivolumab, a mAb directed against PD-1, in comparison with investigator’s choice in recurrent and metastatic HNSCC patients is ongoing (NC102105636). The first results showed an increased overall survival of immunotherapy with nivolumab in comparison with investigators choice (median overall survival 7.5 months versus 5.1 months for nivolumab arm or investigator’s choice, respectively; ref. 83).

Conclusions

HGF/Met-mediated signaling in head and neck cancer is crucial for enhanced proliferation, invasion, and metastasis. HGF/Met signaling clearly correlates with increased recurrence rates and poor patient prognosis. These findings, together with frequent coexpression and mutual interactions with the EGFR, the most prominent RTK in head and neck cancer, provides evidence that this signaling axis is a rationale therapeutic target. The prominent role of HGF in the TME and Met’s effect on immune surveillance and immune activation warrants further investigation in HNSCC. Studies which integrate the effects of HGF/Met signaling on the tumor microenvironment will provide a more complete understanding of the therapeutic value of targeting HGF/Met in HNSCC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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