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.

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 antibody. Clatuzumab, rilotumumab, onartuzumab or crizotinib (NCT01377376 and NCT01662869, respectively) or rilotumumab in gastric cancer (NCT01662869). Importantly, only crizotinib and cabozantinib have received FDA approval for lung adenocarcinoma (19, 20) and RET-positive medullary thyroid carcinoma (21), respectively. Moreover, cabozantinib 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, and circumstance 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 pro-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 nurture a proliferative tumor niche.

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**Figure 1.**

The HGF/Met pathway. The hepatocyte growth factor (HGF) is mainly produced and secreted by the tumor-associated fibroblast (TAF) as an inactive precursor pro-HGF (Step 1; ref. 26). Cleavage of pro-HGF to active HGF is facilitated, among others, by the membrane-anchored enzyme matriptase on the cancer cell surface (Step 2; ref. 34). HGF binding to Met results in a dimerization of two Met receptor molecules (3). Upon dimerization, activation of both receptors is promoted by transphosphorylation at several binding sites (Y1230, Y1234, Y1235; refs. 11, 12). Further tyrosine residues on the C-terminal end (Y1349, Y1356) become phosphorylated, serving as docking sites for downstream adaptor molecules, such as Grb2-associated binding protein 1 (GAB1; Step 4; ref. 16). Importantly, Gab1 as major adaptor molecule for downstream of HGF/Met signaling can bind to Met indirectly via Grb2 (89). Common HGF/Met downstream signaling is mediated by PI3K/Akt/mTOR and STAT3 (Step 5; ref. 16). Activation of these downstream pathways drive transcriptomic changes (Step 6), that mediate a plethora of cancer cell phenotypes (Step 7; refs. 26, 35, 42, 43). The mechanism by which cancer cells engage TAFs to produce pro-HGF is not fully understood (Step 8).
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
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

<table>
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<th>Clinical trial</th>
<th>Phase</th>
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<td>NCT02277197</td>
<td>Ib</td>
<td>Ficlatuzumab plus cetuximab</td>
<td>Recurrent/metastatic HNSCC patients may be cetuximab-exposed or cetuximab-naive (n = 22)</td>
<td>DLT Recruiting</td>
<td>Recruiting June 2018</td>
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<tr>
<td>NCT02277184</td>
<td>Ib</td>
<td>Ficlatuzumab, cisplatin, and intense-modulated radiotherapy</td>
<td>Intermediate- or high-risk LA HNSCC (n = 24)</td>
<td>DLT Recruiting</td>
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<tr>
<td>NCT02205398</td>
<td>Ib</td>
<td>INC280 (capmatinib) plus cetuximab</td>
<td>Metastatic HNSCC Met positive (as defined by IHC and/or FISH) (n = 60)</td>
<td>DLT Recruiting</td>
<td>February 2017</td>
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<tr>
<td>NCT01696955</td>
<td>II</td>
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<td>RR Active, not recruiting</td>
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<tr>
<td>NCT00725764</td>
<td>II</td>
<td>Foretinib</td>
<td>Recurrent/metastatic HNSCC (n = 14)</td>
<td>RR Completed</td>
<td>Stable disease was the best seen response (7/14). Prolonged stable disease (&gt;13 months) in 2/14 patients (45).</td>
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NOTE: Data provided from www.clinicaltrials.gov as of June 2016.
Abbreviations: DLT, dose-limiting toxicity; LA, locally advanced; RR, response rate.
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-directed 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 fclatuzumab 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 fclatuzumab to mitigate the effects of tumor-associated fibroblasts on proliferation, migration, and invasion were recently published (36).
TME-derived HGF was shown to enhance radioresistance and chemoresistance in several cancer entities (53, 54). Also in HNSCC, there is some evidence that HGF/Met signaling might be associated to BclxL expression (55) and radioresistance (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 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 cooccurence 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 efflux in a glycolysis-dependent manner.

Met and antitumor immune response

The interactions between the tumor and the immune system in the tumor microenvironnement 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 potentely 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 bronchialalveolar 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 (NCIT2105636). 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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