

Role of HGF/MET axis in resistance of lung cancer to contemporary management

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Abstract: Lung cancer is the number one cause of cancer related mortality with over 1 million cancer deaths worldwide. Numerous therapies have been developed for the treatment of lung cancer including radiation, cytotoxic chemotherapy and targeted therapies. Histology, stage of presentation and molecular aberrations are main determinants of prognosis and treatment strategy. Despite the advances that have been made, overall prognosis for lung cancer patients remains dismal. Chemotherapy and/or targeted therapy yield objective response rates of about 35% to 60% in advanced stage non-small cell lung cancer (NSCLC). Even with good initial responses, median overall survival of is limited to about 12 months. This reflects that current therapies are not universally effective and resistance develops quickly. Multiple mechanisms of resistance have been proposed and the MET/HGF axis is a potential key contributor. The proto-oncogene MET (mesenchymal-epithelial transition factor gene) and its ligand hepatocyte growth factor (HGF) interact and activate downstream signaling via the mitogen-activated protein kinase (ERK/MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K/AKT) pathways that regulate gene expression that promotes carcinogenesis. Aberrant MET/HGF signaling promotes emergence of an oncogenic phenotype by promoting cellular proliferation, survival, migration, invasion and angiogenesis. The MET/HGF axis has been implicated in various tumor types including lung cancers and is associated with adverse clinicopathological profile and poor outcomes.

The MET/HGF axis plays a major role in development of radioresistance and chemoresistance to platinum, taxanes, camptothecins and anthracyclines by inhibiting apoptosis via activation of PI3K-AKT pathway. DNA damage from these agents induces MET and/or HGF expression. Another resistance mechanism is inhibition of chemoradiation induced translocation of apoptosis-inducing factor (AIF) thereby preventing apoptosis. Furthermore, this MET/HGF axis interacts with other oncogenic signaling pathways such as the epidermal growth factor receptor (EGFR) pathway and the vascular endothelial growth factor receptor (VEGFR) pathway. This functional cross-talk forms the basis for the role of MET/HGF axis in resistance against anti-EGFR and anti-VEGF targeted therapies. MET and/or HGF overexpression from gene amplification and activation are mechanisms of resistance to cetuximab and EGFR-TKIs. VEGF inhibition promotes hypoxia induced transcriptional activation of MET proto-oncogene that promotes angiogenesis and confers resistance to anti-angiogenic therapy. An extensive understanding of these resistance mechanisms is essential to design combinations with enhanced cytotoxic effects.

Lung cancer treatment is challenging. Current therapies have limited efficacy due to primary and acquired resistance. The MET/HGF axis plays a key role in development of this resistance. Combining MET/HGF inhibitors with chemotherapy, radiotherapy and targeted therapy holds promise for improving outcomes.

Keywords: Mesenchymal-epithelial transition factor gene (MET); hepatocyte growth factor (HGF); lung cancer; resistance; epidermal growth factor receptor (EGFR)



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Lung cancer: present-day portrait

Epidemiology

As per 2008 estimates, lung cancer accounts for 1.6 million new cancer cases (13% of all new cancers) and about 1.4 million cancer deaths (18% of all cancer deaths) worldwide, making it the number one cause of cancer related mortality (1). In the United States alone, about 226,160 new lung cancer cases and about 160,340 lung cancer deaths are estimated to occur in 2012 (2). About 85% of all lung cancers are histologically non-small cell lung cancers (NSCLC) and 15% are small-cell lung cancers (SCLC) (3). As per incidence statistics of 1990-2000 and 2000-2009, 40-80% of all NSCLC are localized and the remaining present as either advanced loco-regional disease or with distant metastasis [National Cancer Institute: Surveillance Epidemiology and End Results (NCI: SEER) (seer.cancer.gov)] (4,5). Similarly, 40% of patients with SCLC are staged with limited-stage disease and 60% are staged with extensive-stage disease (6).

Contemporary therapies

The treatment of lung cancer is multifaceted and involves a complex interplay of surgery, radiation, cytotoxic chemotherapy and targeted therapies (5). Accurate staging and stage dependent multidisciplinary care is paramount to improved patient outcomes. The distinction between NSCLC and SCLC guides treatment and prognosis. In NSCLC, although complete surgical resection can result in long-term survival, a significant majority of patients, 50% with stage IB and 70% of stage II, recur (5). Addition of adjuvant platinum-based chemotherapy demonstrated improved survival in early stage NSCLC (7). Radiation therapy is employed for treatment of NSCLC in both the palliative setting and for definitive management of unresectable non-metastatic disease either alone or with concurrent chemotherapy (8-10). Palliative systemic therapy in patients with stage IV NSCLC with either disseminated metastases or malignant effusions is enhanced by molecular characterization including mutational profiles. Cytotoxic chemotherapy forms the backbone of therapy for NSCLC (11). Active agents against NSCLC are platinum compounds (cisplatin, carboplatin), taxanes (paclitaxel, docetaxel), vinorelbine, gemcitabine and pemetrexed (11-13). Multiple targeted agents have also demonstrated activity in metastatic NSCLC. Tumors that exhibit anaplastic lymphoma kinase (ALK) fusions and epidermal growth factor receptor (EGFR) mutations

are treated with ALK inhibitor (Crizotinib) and EGFR inhibitor (erlotinib), respectively (14,15). Targeted agents such as bevacizumab against vascular endothelial growth factor (VEGF) and cetuximab against EGFR in combination with chemotherapy doublets have shown a significant survival benefit in NSCLC (16,17). The management of limited-stage SCLC (LS-SCLC) involves combination chemotherapy with a platinum-based regimen (cisplatin/carboplatin and etoposide/irinotecan/epirubicin/topotecan) in conjunction with concurrent accelerated thoracic radiation therapy, while extensive-stage SCLC (ES-SCLC) is treated with, combination platinum-based chemotherapy (carboplatin and etoposide) alone (18-22). This treatment plan and combined modality approach can be tailored to individuals depending on patient specific factors such as age, performance status, pulmonary functions and comorbidities.

Prognosis

Despite multiple available treatments the overall prognosis of lung cancer remains dismal. The clinical/pathological stage of disease presentation is the single most important prognostic determinant in lung cancer (4). In a large series of patients with NSCLC, median overall survival varied from about 60 months for clinical stage I disease to about 6 months for clinical stage IV disease (4). The 5-year overall survival rate for LS-SCLC and ES-SCLC is 4.8% and 2.3%, respectively. Median disease-free survival in stage I and II NSCLC after resection and adjuvant therapy is about 3 years (23). Response rates and median survival of patients with stage III NSCLC treated with concurrent chemoradiation are 84% and 16.5 months, respectively (24). Patients receiving chemotherapy and/or targeted therapy in metastatic setting have objective response rates of about 35% and median survival of about 12.3 months (16). Similarly, response rates and median overall survival in ES-SCLC is 60% and 11 months, respectively (19).

These figures indicate that current therapies have limited efficacy and even when initial responses are seen, these are short lasting. Tackling primary and acquired resistance to existing therapies is a major challenge in improving patient outcomes in lung cancer.

The MET/HGF pathway: an overview

Structure & function

The proto-oncogene *cMET* (mesenchymal-epithelial transition factor gene) is present on chromosome 7q31

and encodes for a receptor tyrosine kinase (RTK) (25). The MET receptor is a single-pass type I transmembrane disulfide-linked heterodimer protein, made of a short extracellular alpha-chain and a long transmembrane beta-chain (26,27). The beta-chain has an extracellular, a transmembrane and a cytoplasmic domain (26). The cytoplasmic portion of the beta-chain contains the kinase domain of the RTK and also the carboxy-terminal tail with the bidentate multifunctional docking site, essential for intracellular signaling (26,28). HGF or scatter factor (SF) has been identified as the ligand for the MET receptor (29). HGF is a heterodimer, composed of a large alpha-chain and a small beta-chain linked by disulfide bridges (26,30). The ligand HGF dimer binds to the N-terminal portion of MET and causes dimerization of MET receptors (31,32).

The receptor-ligand interaction between MET and HGF, and the resultant dimerization ultimately lead to the activation of the intrinsic kinase activity of MET, which in turn phosphorylates the tyrosine residues at the carboxy-terminal docking site (26). Phosphorylated MET (p-MET) networks with adaptor molecules such as Gab1 (GRB2-associated-binding protein 1), Grb2 (Growth factor receptor-bound protein 2), SRC (Sarcoma non-receptor tyrosine kinase), SHIP-1 (SH2 domain-containing inositol 5-phosphatase 1) and Shp2 (Src homology 2-domain-containing protein tyrosine phosphatase-2) to mediate biological responses (26,33-36). These effector molecules then activate downstream oncogenic signaling that regulates gene expression via the mitogen-activated protein kinase (ERK/MAPK) pathway and the phosphatidylinositol 3-kinase (PI3K/AKT) pathways (*Figure 1*) (26,37,38). In addition to regulating gene expression, these effector molecules can alter cytoskeletal framework and cellular adhesion by modulating actin, catenin, integrins and cadherins via activation of the PAK1 (p21 Activated Kinase) (39).

Role in tumorigenesis

A normally functioning and strongly regulated MET/HGF axis is essential for embryogenesis, liver regeneration and wound healing (26,40-42). However, aberrant MET/HGF signaling promotes survival and migration of cells and can result in tumor development and progression. The cellular responses to activation of the MET/HGF axis support emergence of an oncogenic phenotype by promoting cellular proliferation, survival, migration, invasion and angiogenesis (43-46).

Aberrancy of the MET/HGF axis due to germline or

somatic mutations has been implicated in various tumor types and linked with adverse clinicopathological profile and poor outcomes (26). The most frequent dysregulation in human cancers is due to over-expression of either the ligand HGF/SF or the receptor MET (47,48). Activating mutations of MET gene homologous to mutations seen in other RTKs are also seen but are uncommon (47,49). The MET/HGF pathway has been shown to be involved in carcinogenesis and progression of a variety of tumors such as head & neck squamous cell cancer, breast cancer and colon cancer (48,50-53). Aberrant MET/HGF expression in these tumors has also been shown to correlate with adverse clinicopathological factors such as higher grade, advanced stage and poor survival outcomes (50,54,55). The key role of MET/HGF in carcinogenesis has stimulated interest and investigation of the therapeutic potential of exploiting this axis for treatment of cancer (56,57).

Impact in lung cancer

MET, phosphorylated MET and HGF are highly expressed in both NSCLC and SCLC and appear to correlate with a worse overall survival (58). Although, HGF and MET are expressed in normal lung, their expression is increased in tumor tissue (59). In NSCLC cell lines and tumor tissues MET protein expression correlates with higher pathological tumor stage and worse outcomes (60). HGF induced MET phosphorylation in MET mutant SCLC cell lines promotes proliferation, invasiveness and clonogenic growth and its inhibition counteracts these effects (61). In human clinical samples, MET and p-MET overexpression is seen in 54% and 43% of SCLCs and is a poor prognostic factor (61).

This data has generated immense interest in therapeutic development of HGF/MET inhibition for lung cancer (56). Multiple clinical studies involving inhibition of MET/HGF axis are underway (62).

The MET/HGF axis: signaling pathways & cross-talk

Normal cellular physiology is regulated by a composite array of well-ordered signaling pathways. These complex axes are comprised of ligands, cell surface membrane receptors and intracellular signal transduction molecules. Anomalies in several of such cellular signaling pathways have been implicated in lung carcinogenesis. These aberrations regulate abnormal cellular proliferation, survival, motility and angiogenesis and consequently stimulate tumor growth

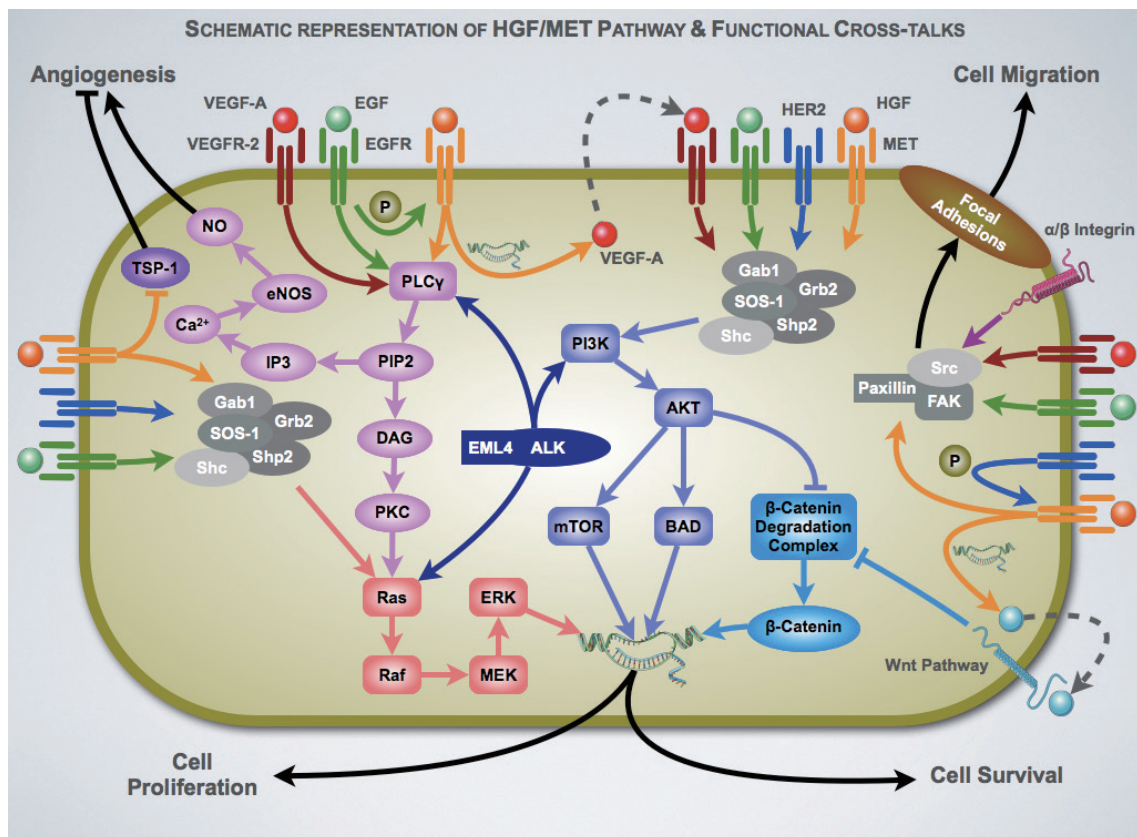


Figure 1 Schematic representation of MET/HGF signaling and functional cross-talk with other pathways. Hepatocyte growth factor (HGF) binds to MET receptor and activates kinase activity which then phosphorylates the docking site and recruits effector molecules [Growth factor receptor-bound protein 2 (GRB2), GRB2-associated-binding protein 1 (GAB1), SRC homology 2 domain-containing phosphatase 2 (SHP2), Son of sevenless-1 (SOS-1) and Sarcoma non-receptor tyrosine kinase (SRC)]. Downstream signaling pathways such as the Mitogen-activated protein kinase (ERK/MAPK) pathway, the Phosphatidylinositol 3 kinase (PI3K-AKT) pathway and the Phospholipase- γ (PLC- γ) pathway are activated. These effector pathways are shared by the epidermal growth factor receptor (EGFR/EGF) pathway, the vascular epidermal growth factor (VEGFR-2/VEGF-A) pathway, the HER2 pathway and the EML4-ALK pathway. The signals affect gene expression and promote cell proliferation and survival, angiogenesis and cytoskeletal alterations resulting in cancer growth and progression. EGFR and HER2 can Trans-phosphorylate and activate MET. MET activation increases transcriptional expression of VEGF-A and WNT ligand and activates VEGF pathway and WNT- β -catenin pathway, respectively. β -catenin increases MET expression.

and progression. Epidermal growth factor receptor (EGFR) pathway, vascular endothelial growth factor receptor (VEGFR) pathway and MET/HGF pathway are pathways of primary interest involved in lung cancer. A substantial functional cross-talk between the MET/HGF axis and these other signaling pathways has been demonstrated both *in vitro* and *in vivo* (63) (Figure 1). These functional relationships and resultant cellular functions in cancer cells are principal mechanisms for cancer progression and therapeutic resistance to targeted therapies.

MET/HGF & EGFR

EGFR is a valid target in NSCLC and plays a crucial role in the biological behavior of these tumors (64). EGFR mutations are seen in 15% to 30% of NSCLCs and are associated with better survival and responsiveness to EGFR inhibition with erlotinib and gefitinib (65,66). There appears to be a reciprocal functional cross-talk between the MET and the EGFR pathways (Figure 1). Activation of EGFR by EGFR ligands can lead to HGF-independent phosphorylation and activation of MET (67). This trans-

phosphorylation indicates close cooperation among the EGFR and MET axis and can in part be responsible for cellular responses to EGFR stimulation. Furthermore, activation of the MET/HGF axis can cause EGFR independent stimulation of EGFR related downstream signaling pathways such as mitogen-activated protein kinase (ERK/MAPK) pathway and the phosphatidylinositol 3 kinase (PI3K-AKT) pathway (26).

MET/HGF & VEGFR

VEGF is a potent angiogenic factor that regulates angiogenesis and has prognostic significance in NSCLC (68). Evidence supports the role of MET/HGF axis as being involved in regulation of angiogenesis and lymphangiogenesis by promoting endothelial cell growth, migration and capillary formation (44,69). The MET/HGF axis also induces expression of VEGF-A by activation of MEK and PI3K pathway (70). HGF signals induce transcriptional activation of VEGF by enhancing promoter activity (71). In addition to the direct effect on endothelial cells and induction of VEGF-A, the axis also suppresses Thrombospondin-1, which is a negative regulator of angiogenesis (72). Akin to the EGFR pathway, the VEGF pathway and the MET/HGF axis also share a common assortment of downstream signaling pathways such as the MAPK pathway and the PI3K-AKT pathway (*Figure 1*).

MET/HGF & other pathways

HER2 overexpression is seen in 30% of patients with NSCLC and portends poor prognosis (73). Both HER2 and MET downstream signaling involves the MAPK and the PI3K pathways and recent data in breast cancer suggests that MET/HGF expression is associated with an increased risk of failure from trastuzumab based therapy in HER2-positive metastatic breast cancer (74).

The Wnt- β -Catenin pathway has been shown to be associated with c-myc and survivin gene expressions and tumor proliferation leading to progression and development of more aggressive tumors in NSCLC (75). There is significant crosstalk between MET/HGF axis and β -catenin signaling. The MET/HGF stimulation increases transcription of WNT ligands which in-turn inhibits β -catenin degradation complex thereby promoting β -catenin nuclear targeting and gene expression (62). Moreover, MET/HGF activation can result in WNT-independent PI3K mediated activation of β -catenin signaling (76,77).

Conversely, an active form of β -catenin increases transcription of MET expression (77).

The *ALK* gene encodes for a receptor tyrosine kinase and the EML4-ALK fusion protein is a driver mutation in 5% to 13% of NSCLCs (14,78). The ALK tyrosine kinase activates downstream signaling pathways such as MAPK and PI3K pathway analogous to MET/HGF and promotes proliferation, migration and inhibits apoptosis (79). These shared signaling pathways and extensive cross-talk are an important consideration while evaluating the possible mechanisms of resistance to targeted therapies. Crizotinib, is a combined ALK and MET inhibitor, and its efficacy in NSCLCs is indicative of feasibility and effectiveness of co-inhibition of cooperating pathways (14,80).

The MET/HGF axis: resistance mechanism to contemporary therapies

Although, our armamentarium of treatment strategies has grown immensely, success has been limited. One of the foremost reasons for failure of contemporary treatments in lung cancer is presence of inherent or development of acquired resistance in cancer cells. Multiple mechanisms of resistance have been identified in lung cancer and MET/HGF axis is one of the pivotal cellular pathways contributing to this therapeutic resistance (*Figure 2*).

Chemotherapy

Platinum based therapy is the backbone of conventional cytotoxic chemotherapy for NSCLC in both adjuvant setting and metastatic setting (7,11). It is also used in chemoradiation as a radiosensitizer (8). Furthermore, platinum agents are employed as first line therapy for both limited-stage and extensive-stage SCLC (18,19). Taxanes are extensively used in treatment of NSCLC (16). Camptothecins such as irinotecan and topotecan show activity in both NSCLC and SCLC and are used either as single agents or in combination with cisplatin/carboplatin (20,21,81,82). Anthracycline drugs such as epirubicin and etoposide are used in treatment of ES-SCLC (21,22). Development of chemotherapy-resistant phenotype is a principal cause of treatment failure in lung cancer.

MET/HGF axis has been frequently implicated in development of chemoresistance in multiple tumor types (*Figure 2*). Cytotoxicity in human glioblastoma tumor cell lines from gamma irradiation, cisplatin, camptothecin, Adriamycin and taxanes has been shown to be partially

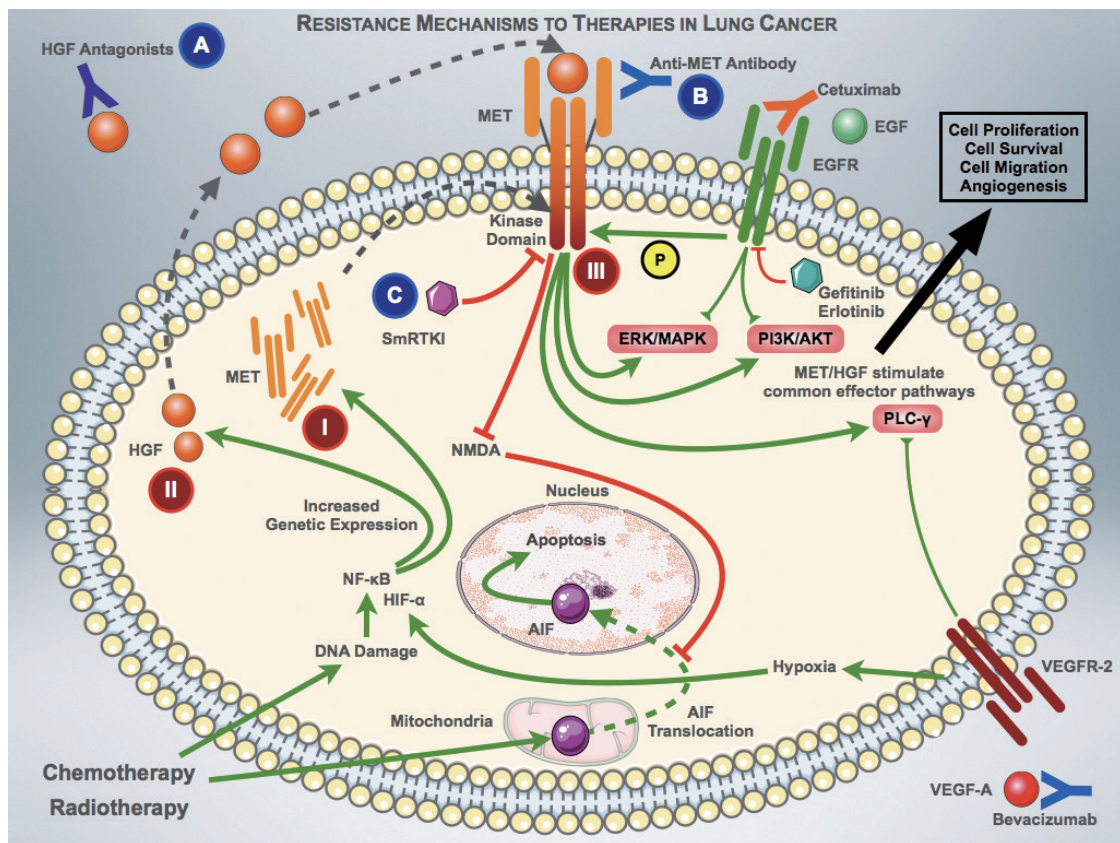


Figure 2 Schematic representation of mechanisms of resistance due to MET/HGF pathway and potential interventional strategies. Chemotherapy and radiotherapy damage DNA which in-turn increases nuclear factor kappa B (NF- κ B) mediated increased expression of HGF and MET. MET [I] and/or HGF [II] over-expression are major mechanisms of resistance due to MET/HGF axis against contemporary therapies. VEGF inhibition can cause hypoxia and increased hypoxia-inducible factor-alpha (HIF- α) mediated HGF and MET expression. Rarely, mutations in the kinase domain [III] can cause constitutional activation of MET signaling and contribute to increased resistance. In gefitinib, erlotinib, cetuximab and bevacizumab resistant tumors, MET/HGF activates oncogenic signaling via Mitogen-activated protein kinase (ERK/MAPK) pathway, the Phosphatidylinositol 3 kinase (PI3K-AKT) pathway and the Phospholipase-C γ (PLC- γ) pathway. The MET/HGF inhibition by HGF antagonists [A], anti-MET antibody [B] or small molecule receptor tyrosine kinase inhibitor (SmRTKI) [C] can help overcome resistance.

abolished by pretreatment with recombinant HGF *in vitro* (83). This cytoprotective effect from HGF is a result of inhibition of apoptosis, mediated by MET/HGF signaling that activates PI3K-AKT pathway (83). Studies have also demonstrated that HGF transfected Chinese hamster ovary cells are resistant to treatment with cytotoxic DNA damaging agents such as Adriamycin and irinotecan. The increased cell viability was the result of decreased apoptotic cell death induced by these agents indicating the protective effect of HGF against programmed cell death following DNA damage (84). Rhabdomyosarcoma cell lines overexpressing HGF and MET exhibit increased survival

even on exposure to chemotherapy (85).

Multiple mechanisms of MET/HGF induced-chemoresistance have been proposed (Figure 2). Most cytotoxic agents promote apoptosis through programmed cell death (86). The MET/HGF axis down-regulates the expression of anti-apoptotic protein Bcl-XL (87). MET/HGF also inhibits N-methyl-d-aspartate (NMDA)-induced activation of caspase 3 and NMDA-induced apoptosis-inducing factor (AIF) translocation from mitochondria to nucleus thereby preventing NMDA-induced cell death in cultured hippocampal neurons (88). Similarly, HGF suppresses AIF expression through activation of FAK and

induces cisplatin resistance in lung cancer cell lines (89). Additionally, DNA damaging agents such as Adriamycin, camptothecins, cisplatin and etoposide induce translocation of AIF, a mitochondrial effector of apoptotic cell death, from mitochondria to nucleus (90). A possible mechanism for this translocation is activation of poly (ADP-ribose) polymerase-1 (PARP-1), which protects the genome by functioning as a DNA damage surveillance network, by DNA damaging agents (91). PARP-1 activation induces translocation of AIF which induces caspase-independent chromatin condensation and DNA fragmentation (91-93). AIF-mediated apoptotic death is critical for treatment effect in NSCLC cell lines (94). MET/HGF mediated inhibition of AIF is therefore a potential mechanism for resistance to cytotoxic chemotherapy in lung cancer.

This data illustrates a vital role of the MET/HGF axis in conferring chemoresistance to common cytotoxic chemotherapy in lung cancer. MET/HGF inhibition can therefore be used strategically to overcome chemoresistance and improve response rates and survival outcomes with conventional chemotherapy.

Radiation

Radiotherapy forms the cornerstone of combined-modality therapy for unresectable stage III NSCLC and LS-SCLC (19,95). In NSCLC, complete response (CR) rate with concurrent chemoradiotherapy is about 40% and 5-year OS is about 16% (8). Similarly, SCLC, which is radiosensitive tumor, CR rate with concurrent chemoradiation is about 50% and median OS is about 2 years (96). Radiotherapy resistance is a probable mechanism for these sub-optimal response rates and recurrent disease despite aggressive treatment.

Radiation causes DNA damage and the resultant cellular radiosensitivity in cancers is a function of activation of apoptotic programmed cell death (97,98). Therefore, acquired cellular insensitivity to radiation therapy is in part caused by activation of anti-apoptotic signals such as the PI3K/AKT pathway (99). Notably, the PI3K/AKT pathway can also be activated by MET/HGF signaling (26,99). Additionally, radiation induced hypoxia can cause transcriptional activation of *MET* proto-oncogene (100). This effect is mediated by initial recognition of radiation-induced DNA damage by the ATM protein kinase, which in turn activates nuclear-factor kappa B (NF- κ B) (101). The NF- κ B binds to the *MET* promoter and increases MET expression (*Figure 2*) (101).

Multiple studies have corroborated stimulation of MET/HGF axis by radiation. In human malignant glioma cell lines, radiation exposure leads to a dose-dependent increase of HGF levels and development of radioresistance (102). In lung cancer and pancreatic cell lines, gamma-radiation leads to substantially increased MET expression and phosphorylation (101,103). HGF ligand in presence of such upregulated MET receptors abolishes radiation-compromised cell migration and can lead to emergence of metastatic phenotypes (103). The overexpressed MET receptor in such irradiated cell lines possesses constitutively activated kinase activity and phosphorylation as well as increased sensitivity to HGF ligand (101). These results were reproduced in a panel of neuroblastoma cell lines, which when exposed to radiation showed enhanced HGF mRNA expression and MET receptor amplification (104). Both ligand-dependent and ligand-independent phosphorylation of MET was seen in such cell lines (104). Similarly, HGF and MET overexpression has also been shown to increase survival in rhabdomyosarcoma cell lines exposed to radiotherapy (85). This data illustrates the importance of MET/HGF in development of acquired resistance to radiotherapy (101).

The presence of residual disease after radiation therapy and disease relapse after initial responses indicates emergence of radio-resistant cancer cells and MET/HGF appears to play a significant role in this phenomenon. Radiotherapy in combination with MET/HGF inhibition, used as a radiosensitizer, is hence a promising therapeutic partnership.

Targeted therapies

Systemic palliative management of metastatic NSCLC is individualized based on mutational profile of the tumors. The molecular characterization to date involves evaluating for mutations against which, efficacious targeted therapies are available and include EGFR mutations or expression (erlotinib, gefitinib, and cetuximab) and EML/ALK4 rearrangement (crizotinib) (14,15,17). Bevacizumab in combination with chemotherapy improves survival in adenocarcinomas (16). However, responses are still restricted and disease progression occurs invariably. Despite the sound scientific rationale of anti-angiogenesis, the 2 months improvement in overall survival with addition of bevacizumab to chemotherapy in the E4599 trial was not duplicated in the AVAiL trial (16,105). Similarly, increase in response rates to addition of cetuximab in EGFR expressing

tumors was only about 20% and median OS improved by about 1.2 months (17). Although, gefitinib therapy in WJTOG3405 trial was associated with a response rate of 60%, the progression-free survival was only increased by 3 months (106). As is evident from above data, a majority of patients initially respond to targeted therapies but subsequently develop disease progression possibly as a result of emergence of acquired resistance and alterations in the MET/HGF signaling cascade appear to be a key player in this phenomenon (Figure 2).

EGFR inhibition resistance

The functional cross-talk between the EGFR and MET pathway is clinically significant and is probably responsible for both primary and acquired resistance against EGFR directed therapies in lung cancer. It has been established that dual receptor activation of cell lines co-expressing EGFR and MET, results in increased proliferation of such cell lines (107).

Although, EGFR expression is common in lung cancers, only a minority of patients respond to cetuximab due to presence of primary resistance and most of those who respond do so only transiently due to acquired resistance. Increased MET/HGF activity is a possible mechanism of this resistance. Cells previously treated with cetuximab, when cultured with HGF exhibit MET phosphorylation and revamped stimulation of downstream effector pathways (MAPK and PI3K/AKT) which restore cell proliferation and prevents apoptosis (107). Similarly, patient-derived xenografts from NSCLC demonstrated that MET overexpression from gene amplification and activation is a major mechanism of primary cetuximab resistance (108). The HGF induced resistance to cetuximab is seen regardless of EGFR gene status in both wild-type and mutant EGFR cells (109).

Small molecule EGFR tyrosine kinase inhibitor such as gefitinib, are effective in NSCLC and act via reduction of ErbB-3 mediated PI3K/AKT growth signaling (110). Both primary and secondary resistance mechanisms to EGFR-TKIs in NSCLC have been described (111). Although, secondary T790M mutation in EGFR is the most common cause (50%) of acquired resistance to EGFR-TKIs, multiple other pathways are also involved (112,113). MET/HGF has been identified as a novel mechanism of resistance to EGFR-TKIs (114). Only about 10% of unselected NSCLC patients respond to EGFR-TKIs and strong MET membrane immunoreactivity has been shown to

be associated with progressive disease and shorter time to progression and thus may be regarded as marker of primary gefitinib resistance in NSCLC patients (115). MET/HGF signaling activates PI3K/AKT pathway and may contribute to gefitinib resistance using this downstream effector pathway (26). Study with a gefitinib-sensitive lung cancer cell line demonstrated that acquired resistance to gefitinib develops as a result of focal amplification of the MET proto-oncogene (116). MET amplification is seen in 22% of lung cancer specimens that develop resistance to gefitinib or erlotinib possibly due to re-activation of PI3K pathway (116). Similarly, HGF-mediated MET activation has also been shown to confer gefitinib resistance in lung adenocarcinomas (117).

VEGFR inhibition resistance

Bevacizumab in combination with chemotherapy is used in NSCLC and improves response rates but these responses are not universal and often short lived (16). The MET/HGF axis promotes angiogenesis by promoting endothelial cell growth, migration and capillary formation, induces expression of VEGF-A and suppresses Thrombospondin-1 thereby contributing to resistance against VEGF inhibition (44,70,72). Hypoxia promotes transcriptional activation of MET (100). Dual inhibition of MET and VEGFR-2 has demonstrated strong inhibition of tumor growth and tumor angiogenesis in xenograft models (118).

Others

Multiple molecular pathways have been shown to be involved in carcinogenesis of lung neoplasms. The limited single-agent clinical activity demonstrated by several targeted agents in lung cancer begs the question that, are there intrinsic resistance pathways that overcome single pathway inhibitions? Understanding these resistance mechanisms is essential to hypothesize rational combinations that would enhance cytotoxic effects and exploit the full potential of targeted therapies.

HER2 expression is seen in 30% of NSCLCs and 25% of SCLCs and is associated with poor prognosis (73,119). HER2 overexpression in NSCLC is also a marker for multidrug resistance (120). Anti-Her2 therapy has shown significant anti-tumor effect against NSCLC, both *in vivo* and *in vitro* (121,122). However, multiple trials of trastuzumab in NSCLC have failed to show any significant clinical activity (123,124). MET expression has been shown

to contribute to trastuzumab resistance in breast cancer and could be a factor contributing to suboptimal responses to trastuzumab in NSCLC (124,125). Anti-Her2 therapy could be therapeutic strategy in NSCLC due to significant expression seen in NSCLC and also since HER2 expression can contribute to angiogenesis by enhancing VEGF secretion (126).

Histone deacetylase 1 (HDAC1) expression in lung cancer is associated with poor 5-year disease-free survival rate (127). Histone deacetylase inhibitors such as vorinostat can induce tumor cell growth arrest, differentiation and apoptosis. A phase II trial of vorinostat carboplatin plus paclitaxel showed increased response rate with vorinostat (128). HDAC regulates chromatin remodeling and down-regulates transcription of miR-449, which is an inhibitor of MET expression (129). MET/HGF overexpression can therefore potentially lead to resistance against HDAC inhibitors.

Future

General

Treating lung cancer has been a daunting task. A myriad of therapies are available but outcomes remain below par. Molecular characterization of lung cancer has taught us that this disease is heterogeneous and that molecular pathology guides prognosis and response to therapy. The MET/HGF pathway has been identified as a novel driver of lung carcinogenesis. Besides its role as a mitogen, motogen and angiogen, MET/HGF also plays a key role in both primary and acquired resistance to contemporary therapies. The potential role of the MET/HGF axis as a predictive and prognostic marker needs further exploration.

Potential for therapeutic interventions

MET/HGF axis has recently emerged as a potential therapeutic target and multiple agents including monoclonal antibodies to either the MET receptor or HGF and small molecular tyrosine kinase inhibitors against MET RTK are being investigated (130). Recognition of the role of MET/HGF axis as a potent resistance mechanism has steered interest in development of therapeutic strategies using MET/HGF inhibition to overcome resistance to contemporary therapies in lung cancer (131).

Inhibition of the MET receptor by gene transfer in tumor cell lines inhibits the cytoprotective anti-apoptotic effect of HGF against DNA-damaging agents such as

gamma irradiation, cisplatin, camptothecin, Adriamycin and taxanes (83). Combination of MET/HGF inhibitors with chemotherapy can possibly improve tumoricidal activity of cytotoxic chemotherapy and improve response rates and outcomes.

Viability of irradiated radio-resistant tumor cell lines treated with MET inhibitor is reduced dramatically after irradiation as a result of increased caspase-3 mediated apoptosis (101). Moreover, treatment with MET inhibitor decreased proliferative capacity of cells that survived irradiation (101). Xenografts using these cell lines showed a significant decrease in tumors when treated with radiation and MET inhibition as compared to radiation alone (101). Recombinant HGF antagonist can block enhanced malignant potential of tumors by radiation-induced MET/HGF amplification thereby improving the efficacy of radiotherapy (103). Similarly, targeting endogenous expression of HGF and MET in human malignant glioma cells and xenografts sensitizes cells to gamma-radiation and enhances radiation-induced caspase-dependent cytotoxicity (132). These studies provide proof of concept regarding synergistic antitumor response of radiation and MET/HGF inhibition (132).

MET inhibition by selective tyrosine kinase inhibitors or downregulation of MET expression by a specific siRNA has been shown to abolish the HGF induced cetuximab resistance in cell lines and restore growth-inhibitory effects of cetuximab (107). MET knockdown not only restores cetuximab resistance but also reduces EGF stimulated EGFR phosphorylation (108). Cetuximab resistance due to HGF can also be abrogated by treatment with anti-HGF neutralizing antibody (109). HGF mediated resistance to gefitinib in lung cancer cell lines can be reversed by down-regulation of MET expression by MET-specific siRNA (117). HGF induced hyposensitivity to EGFR-TKI can be abrogated by treatment with anti-HGF neutralizing antibody, HGF antagonist or MET-TKI (114). A phase II trial of ARQ197 plus erlotinib, a small molecule inhibitor of MET receptor, in NSCLC showed evidence of activity with this combination (133). As a result of this trial, MARQUEE, a phase III, randomized, placebo-controlled study has been initiated in NSCLC patients with the rationale that dual inhibition of MET and EGFR may overcome resistance to EGFR inhibitors (134). Therapy with MET/HGF inhibitors and EGFR inhibitors hold promise for select patients who either fail to respond to anti-EGFR therapy or acquire resistance after an initial response.

Combined MET and VEGF inhibition has been shown to be effective in inducing tumor regression and also in

overcoming HGF-induced EGFR-TKI resistance (118,135). Valproic acid, a HDAC inhibitor, has been shown to inhibit HGF expression and HGF mediated invasion in hepatocellular carcinoma cells (136). Additionally, SCLC cell lines exposed to HDAC inhibitors and topoisomerase inhibitors showed an additive-synergistic response and decreased cell viability (137). Triple target inhibition using combination of MET, EGFR and Her2 inhibitors has illustrated that even though each drug alone was not very effective, the combination resulted in a synergistic inhibition of proliferation and more cytotoxicity (138). MET inhibition has been shown to restore sensitivity to anti-Her2 in cell lines (138). The existing data provides a conceptual framework for combining various targeted therapies with MET/HGF inhibitors to improve tumor kill.

MET/HGF has the potential to emerge in future as a chief molecular target that can be manipulated pharmacologically to increase tumor-cell cytotoxicity. Combined treatments of MET/HGF inhibitors and other contemporary therapies in selected lung cancer patients could result in pronounced clinical benefits.

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