Hepatocyte Growth Factor/cMET Pathway Activation Enhances Cancer Hallmarks in Adrenocortical Carcinoma

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Abstract

Adrenocortical carcinoma is a rare malignancy with poor prognosis and limited response to chemotherapy. Hepatocyte growth factor (HGF) and its receptor cMET augment cancer growth and resistance to chemotherapy, but their role in adrenocortical carcinoma has not been examined. In this study, we investigated the association between HGF/cMET expression and cancer hallmarks of adrenocortical carcinoma. Transcriptomic and immunohistochemical analyses indicated that increased HGF/cMET expression in human adrenocortical carcinoma samples was positively associated with cancer-related biologic processes, including proliferation and angiogenesis, and negatively correlated with apoptosis. Accordingly, treatment of adrenocortical carcinoma cells with exogenous HGF resulted in increased cell proliferation in vitro and in vivo while short hairpin RNA–mediated knockdown or pharmacologic inhibition of cMET suppressed cell proliferation and tumor growth. Moreover, exposure of cells to mitotane, cisplatin, or radiation rapidly induced pro-cMET expression and was associated with an enrichment of genes (e.g., CYP450 family) related to therapy resistance, further implicating cMET in the anticancer drug response. Together, these data suggest an important role for HGF/cMET signaling in adrenocortical carcinoma growth and resistance to commonly used treatments. Targeting cMET, alone or in combination with other drugs, could provide a breakthrough in the management of this aggressive cancer. Cancer Res; 75(19); 1–12. © 2015 AACR.

Introduction

Adrenocortical carcinoma is a rare endocrine malignancy that originates in the adrenal cortex. Adrenocortical carcinoma has poor prognosis with an estimated recurrence rate of 60% to 70% after resection of tumors confined to the adrenal gland and the 5-year survival rate for patients presenting with stage IV disease of about 15% (1–3). While surgery remains the best option for adrenocortical carcinoma patients presenting with localized disease, surgical resection is often not feasible in patients with advanced/recurrent disease, and systemic chemotherapy is often used. The response rate with the current first-line chemotherapy regimen (etoposide, doxorubicin, and cisplatin with mitotane) is only 23%, and median time to disease progression is about 6 months (4). Unfortunately, there are no approved second-line regimens, and patients are often referred to clinical trials using agents or regimens with proven efficacy after failing first-line treatment. Similarly, adrenocortical carcinoma has limited response to external beam radiation, and radiotherapy is mostly used as a palliative measure (5, 6). Thus, there is an urgent need to identify clinically actionable molecular pathways driving adrenocortical carcinoma tumorigenesis and tumor progression.

To develop more effective and less toxic treatments for adrenocortical carcinoma, much of the research over the past two decades has focused on understanding the molecular pathways involved in adrenocortical carcinoma. It is well accepted that insulin-like growth factor-2 (IGF2) is overexpressed in most cases of adrenocortical carcinoma. IGF2 promotes tumor cell growth through IGF1 receptor–mediated downstream activation of the protein kinase B (AKT)/mTOR pathway, but does not seem to be the major driver of adrenocortical carcinogenesis (7). Clinical studies using inhibitors of IGF1R/mTOR signaling have revealed...
minimal tumor responses (8). We hypothesized that other regulatory pathways are simultaneously active in adrenocortical carcinoma, leading to invasive behavior and treatment resistance. cMET has been reported to be expressed in normal adrenal tissue (9), and the cMET signaling pathway (Fig. 1A), which is activated through binding to HGF, is critical in tumor progression/invasiveness and therapy resistance in multiple malignancies (10–13). However, the roles of HGF and cMET in adrenocortical carcinoma have not been evaluated. We therefore examined the potential contribution of the HGF/cMET pathway to cancer hallmarks in adrenocortical carcinoma as an essential step toward exploration of the utility of drugs targeting this pathway.

**Materials and Methods**

**External adrenocortical carcinoma databases**

Transcriptomic profiles of adrenocortical carcinoma datasets GSE10927 and GSE49278 were downloaded from the Gene Expression Omnibus databases (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE10927; http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE49278). The GSE10927 dataset included 10 normal adrenal cortex samples, 22 adrenocortical adenoma samples, and 33 adrenocortical carcinoma samples (14), and the GSE49278 dataset included 44 adrenocortical carcinoma samples (15). The downloaded GSE10927 raw data from Affymetrix HG U133 plus 2 arrays and the GSE49278 raw data from Affymetrix Human Gene 2.0 ST arrays were analyzed using Nexus Expression 3.0 software (BioDiscovery) and gene set enrichment analysis comparing the MET mRNA gene expression profiles of the highest MET expression quartile with those of the two lowest MET expression quartiles. The widths of the links and the relationship of the biologic processes to cancer hallmarks were determined using Z scores and illustrated in a Circos plot (16, 17). Genes from the GSE10927 dataset with significant changes in expression relative to that in noncancerous adrenocortical tissue (P ≤ 0.01, absolute value of log ratio >0.1, pool size for intensity-based pooling 100,000) are presented as heatmaps (Supplementary Fig. S1) and are listed in Supplementary Table S3.

**Patient tissue samples**

Adrenocortical tissue samples were collected from available specimens in our pathology department and analyzed according to a protocol approved by the Institutional Review Board (IRB) of The University of Texas MD Anderson Cancer Center (Houston, TX). We measured serum HGF in 22 adrenocortical carcinoma patients and 7 healthy controls (Supplementary Table S1). Two tissue microarrays were constructed from core samples in duplicates and prepared by the Biospecimens Core Facility at MD Anderson Cancer Center. The first tissue microarray (TMA) contained duplicate cores from 13 adrenocortical carcinoma patients and 7 adrenal adenoma samples. The second TMA included 55 evaluable adrenocortical carcinoma cores (from 28 chemotherapy-naive adrenocortical carcinoma patients and constructed as duplicate cores from each subject) and 15 adrenal adenoma samples (from 15 patients with adrenal adenomas that were constructed as single cores from each patient to serve as control; Supplementary Table S2). Tissues and serum samples were collected and frozen prospectively, after we had obtained patients’ written informed consent to participate in our research according to a protocol approved by our IRB, or were obtained retrospectively from our institutional tissue bank. A waiver of the...
requirement for informed consent was granted by the IRB for inclusion of specimens that were retrospectively obtained. In all cases, the diagnosis of adrenocortical carcinoma was confirmed by board-certified pathologists based on Weiss scores ≥ 3 (18).

Cell lines and reagents

The NCI-H295R human adrenocortical carcinoma cell line was obtained from the ATCC (catalog no. CRL-2128). NCI-H295R cells were grown in DMEAM–Ham F12 medium supplemented with 5% Nu-Serum I (BD Biosciences), ITS (BD Biosciences; 0.00625 mg/mL insulin, 0.00625 mg/mL transferrin, 6.25 ng/mL selenium, 1.25 mg/mL bovine serum albumin and 0.00535 mg/mL linoleic acid), and antibiotic–antimycotic solution (Corning Cellgro; 100 IU/mL penicillin, 100 μg/mL streptomycin, and 250 ng/mL amphotericin B). Cell viability was measured using the MTT assay, as described previously (19). The total number of live cells was determined by multiplying the number of cells counted with a Coulter counter by the percentage of live cells in the cell population as determined by Trypan blue dye exclusion. For radiation experiments, H295R cells were irradiated at room temperature with a Mark I 137Cs irradiator (JL Shepherd & Associates) at a dose rate of 3.5 Gy/minute (8Gy). Protein lysates were collected at different time points after irradiation (1, 3, 6, 12, and 30 hours). Protein level of phospho-STAT3, phospho-ATF2, and phospho-cMET was evaluated by Western blot analysis.

Protein analysis

Protein level of cMET and phospho-cMET was evaluated by Western blot analysis. Protein level of phospho-cMET and phospho-cMET was evaluated by Western blot analysis. Protein lysates were collected at different time points after irradiation (1, 3, 6, 12, and 30 hours). Protein level of cMET and phospho-cMET was evaluated by Western blot analysis. Protein lysates were collected at different time points after irradiation (1, 3, 6, 12, and 30 hours). Protein level of cMET and phospho-cMET was evaluated by Western blot analysis.

Immuno histochemical analysis

Tissue microarray slides were then stained with antibodies against cMET (Cell Signaling Technology #8198; 1:200 dilution), anti-cMET phosphorylated at Y1234/1235 at Y1234/1235 (Cell Signaling Technology #8198; 1:200 dilution), against cMET (Cell Signaling Technology #8198; 1:200 dilution), and against cMET (Cell Signaling Technology #8198; 1:200 dilution) according to a standard immunohistochemistry protocol. Slides were also stained for markers of cell proliferation (Ki-67), tumor vascularity (CD34), and apoptosis (cleaved caspase-3) at the core laboratory of the MD Anderson Department of Pathology. After staining, the IHC slides were analyzed and quantified objectively using an ACIS III Image Analysis System (Dako Corporation). The intensity of immuno histochemical (IHC) staining within tumor areas was determined by the ACIS III Image Analysis System. IHC staining intensity values were used for statistical analyses and graph preparation. IHC staining was additionally analyzed by pathologists at MD Anderson Cancer Center. Representative photomicrographs were obtained using Dako ACIS and Olympus microscopes.

Serum HGF measurement

Patients' serum samples were obtained by centrifuging blood samples at 900 × g for 15 minutes at 4°C. Human HGF levels were measured by enzyme-linked immunosorbent assay according to the manufacturer's protocol (Sigma-Aldrich).

Protein analysis

All protein analyses were performed using lysates from whole cell pellets or human tumor samples in radioimmunoprecipitation assay buffer, as previously described (19). Protein level of cMET and phospho-cMET was evaluated by Western blot analysis. Protein level of phospho-STAT3, phospho-ATF2, and phospho-cJUN was evaluated by ELISA-based xMAP multiplex immunoanalysis as described by the manufacturer (EMD Millipore). Antibodies against cMET and phospho-cMET (Y1234/1235) were obtained from Cell Signaling Technology. Secondary antibodies goat anti-mouse IgG (1:10,000 dilution in 1× TBST solution containing 3% BSA) and goat anti-rabbit IgG (1:10,000 dilution in 1× TBST solution containing 3% BSA) were obtained from Sigma-Aldrich.

RNA analysis

Total RNA was isolated from treated cells using TRRzol reagent (Invitrogen Life Technologies) by following the manufacturer's protocol, as previously described (19). The Qiagen RNeasy Mini Kit was used to increase RNA purity and remove residual genomic DNA. Single-stranded complementary DNA from RNA samples (1 μg of total RNA) was generated using the iScript cDNA synthesis kit (Bio-Rad). Relative gene expression was determined by real-time quantitative PCR using an Applied Biosystems StepOnePlus Real-Time PCR system (Invitrogen) and iQ SYBR Green Supermix reagents (Bio-Rad). The sequences of the primers used for the relative gene expression analysis of MET (long isoform a, accession number NM_001127500) were 5′ cagcagtgcgcagtggtgagtcg (forward) and 5′ gaattcttctggtagagagcg (reverse). The expression level of the β-actin housekeeping gene, ACTB, was used as the internal control and analyzed in each experiment for normalization. The sequences of the primers used for ACTB (accession number NM_00111013.1) were 5′ gatgaggaggaaggtggagcg (forward) and 5′ agaacgttggtgccag (reverse). Relative changes were calculated using the ΔΔCt formula.

Animal models

For xenografting in vivo experiments, we generated H295R- cMET-KD cells with decreased cMET expression by lentiviral infection with cMET shRNA. As control cells, we generated H295R-GFP-KD cells by lentiviral infection with GFP shRNA. H295R-cMET-KD or H295R-GFP-KD cells (4 × 106 cells) in 100 μL with 50% Reduce Growth Hormone Matrigel (BD Biosciences) were injected into the right flank of male Nu/Nu mice (n = 5 mice per group). XL-184 (cabozantinib, a small-molecule tyrosine kinase inhibitor targeting VEGFR and cMET), which is property to the NCI Collaborator Exelixis, Inc., was provided through the Cancer Therapy Evaluation Program. For in vivo experiments with cabozantinib, NCI-H295R cells (6 × 106 cells) in 100 μL with 50% Reduce Growth Hormone Matrigel (BD Biosciences) were injected into the right flank of male Nu/Nu mice. After one to two weeks of cells inoculation, when the tumors reached 5 mm in diameter, xenografted mice were randomized into cabozantinib treatment (30 mg/kg/d; ref. 20) and placebo groups (n = 6 mice per group). The selected dose of cabozantinib experiments is in line with similar published data in mouse experiments (20). Tumor growth was measured by means of tumor volume. Measurements of tumor volume were taken every week or two weeks, and volumes were estimated with this formula: length × width2/2. All animal experiments were conducted in accordance with American Association for Laboratory Animal Science regulations and the approval of The University of Texas MD Anderson Cancer Center Institutional Animal Care and Use Committee.

Statistical analysis

Statistical differences were assessed with Student t-test or the Mann–Whitney U test, as appropriate. For experiments involving more than two groups, we used the one-way analysis of variance;
post hoc intergroup comparisons were performed using the Kruskal–Wallis test, and the Bonferroni correction was used to account for false discovery. All data are reported as means ± 95% confidence intervals. All results were considered statistically significant when P value was <0.05 except when the Bonferroni correction was applied. GraphPad Prism version 5.0d software was used for the statistical analysis and data presentation.

Results
High expression and activation of HGF/cMET signaling in adrenocortical carcinoma patients

Our analysis of transcriptomic profiles of an adrenocortical carcinoma patient cohort (dataset GSE10927, National Center for Biotechnology Information; ref. 14) revealed significant upregulation of MET mRNA in adrenocortical carcinoma samples compared with adrenal adenoma and normal adrenocortical tissue samples (Supplementary Fig. S1; Supplementary Table S3). This finding was confirmed using real-time PCR analysis of an independent set of adrenocortical carcinoma and adrenal adenoma samples obtained at our institute following IRB approval (Supplementary Fig. S2). Immunohistochemical analysis of two independent tissue microarrays and Western blot analysis results further demonstrated a significant adrenocortical carcinoma-specific elevations of HGF and total cMET protein levels, and activation of cMET signaling, as seen by phosphorylation at the Y1234/1235 sites (Figs. 1 and 2 and Supplementary Table S1). We were also able to detect HGF in the culture medium of human adrenocortical carcinoma cell line NCI-H295R at a concentration of 497.2 pg/mL. This suggests a potential autocrine loop in adrenocortical carcinoma.

Increased HGF/cMET signaling is associated with enhanced proliferation, angiogenesis, tumor growth, and reduced apoptosis in adrenocortical carcinoma

To validate the significance of HGF/cMET activation in adrenocortical carcinoma, we evaluated the correlation between HGF/cMET activation and proliferation, promotion of angiogenesis, and apoptosis. Tumor tissue microarray sections (55 adrenocortical carcinoma scores from 28 therapy-naive patients and 15 adenoma cores from 15 individual patients) stained for HGF, cMET, and phosphorylated cMET antibodies were positively correlated with cell proliferation marker (Ki-67) and tumor vascularity (CD34 staining; Fig. 5 and Supplementary Fig. S4) and negatively correlated with apoptosis (cleaved caspase-3 staining; Supplementary Fig. S4). To evaluate HGF’s effect on adrenocortical carcinoma growth and viability, we added recombinant human HGF into the culture medium of NCI-
H295R adrenocortical carcinoma cells. Recombinant HGF significantly stimulated in vitro NCI-H295R cell viability (Fig. 7A) and proliferation (Supplementary Fig. S5). These findings are in concordance with the fact that HGF activates cMET, leading to enhanced cancer cell proliferation and metastatic potential, and HGF activation of cMET is associated with poor prognosis in a variety of malignancies (10–12, 24–28).

Only a small percentage of adrenocortical carcinoma patients respond to currently available systemic therapy (4–6). Genes associated with resistance to or metabolism of cisplatin, etoposide, and doxorubicin were significantly enriched in adrenocortical carcinoma patients' tumor tissues with high MET expression (Fig. 4; Supplementary Tables S11 and S12). Interestingly, we found that cisplatin and mitotane (two key components of first-line chemotherapy for advanced adrenocortical carcinoma), and radiation treatment induce cMET expression in NCI-H295R cells, as manifested by a rise in pro-cMET (Fig. 6A–C). Further bioinformatics analysis demonstrated overexpression of genes related

Figure 3.
cMET is associated with enhancement of cancer hallmarks in adrenocortical carcinoma. A, Circos plot of the association between significantly increased biological processes ($P < 0.05$; see also Supplementary Table S2) and cancer hallmarks (symbols and color-coded labels are indicated on the right) upon MET overexpression. The widths of the connectors represent the absolute values of the Z scores of the biological processes. Bar graphs on the right illustrate the enrichment of some important biological processes. B, the left panel shows a heatmap of changes in gene expression associated with high MET expression. The right panel shows a Venn diagram of dataset GSE10927 microarray data from pretreatment tumor biopsy samples of adrenocortical carcinoma patients; representative genes that were significantly upregulated or downregulated upon MET overexpression ($P < 0.05$, log ratio $> 0.1$) are indicated.
to drug metabolism (Fig. 6D and E; Supplementary Table S10) in patients with high MET expression.

To further investigate the functional role of cMET in adrenocortical carcinoma cell biology, we generated a NCI-H295R adrenocortical carcinoma cell line with decreased cMET expression by knockdown using cMET-targeted shRNA (H295R-cMET-KD). Knockdown of cMET mRNA significantly decreased in vitro cell proliferation (Fig. 7B) and induced cell-cycle arrest (Fig. 7C and D). To further validate the role of cMET signaling in adrenocortical carcinoma tumor growth and progression, we established an adrenocortical carcinoma in vivo xenograft mouse model using H295R-cMET-KD cells. Our results confirmed that cMET knockdown significantly decreased \( P < 0.05 \) tumor growth (Fig. 7E and F). Moreover, in vivo inhibition of cMET by cabozantinib (a commercially available small-molecule tyrosine kinase inhibitor with potent activity toward cMET; ref. 29) significantly \( P < 0.001 \) reduced tumor growth (Fig. 7G and H). Our results also show that stable knockdown of cMET mRNA significantly reduced both mitochondrial respiration and glycolytic metabolism (Supplementary Fig. S6). Thus, adrenocortical carcinoma tumor growth is dependent, at least in part, on cMET signaling, and cMET inhibition is likely to have a role in treatment of advanced adrenocortical carcinoma.

Discussion

We found that HGF/cMET are expressed at a higher level in adrenocortical carcinoma than in adrenal adenomas and normal cortex. Moreover, activation of HGF/cMET appears to enhance adrenocortical carcinoma growth, tumor-related angiogenesis, chemotherapy resistance, and cell survival. Therefore, our data suggest that cMET may be a valuable therapeutic target for adrenocortical carcinoma.

The field of adrenal neoplasia has achieved important milestones during the past 25 years, including the discovery of major genetic alterations and molecularly characterizing adrenal cortical cancer genomic profiles (15, 30). However, adrenocortical carcinoma is considered an aggressive malignancy with limited response to chemotherapy (1, 4–6, 31, 32). Therefore, deciphering the mechanisms driving adrenal cortical tumorigenesis as well as identifying the vulnerabilities of this aggressive type of cancer remains a challenge in this field (15, 30, 33). Most adrenocortical carcinomas show IGF2 overexpression with the possible role of...
the AKT/mTOR pathway as a downstream effect promoting tumor cell growth, but with minimal effect in adrenocortical carcinogenesis (7). Clinical studies to determine the effect of blocking mTOR signaling have revealed minimal tumor responses (8). We hypothesized that other signaling pathways are simultaneously active in adrenocortical carcinoma, leading to invasiveness and treatment resistance. HGF activates cMET in an autocrine and paracrine fashion, leading to enhanced cancer cell proliferation and metastatic potential and associated with poor prognosis in a variety of malignancies (10–12, 24–27). For the first time, we report that the expression of HGF/cMET is high in adrenocortical carcinoma and that cMET activation is associated with adrenocortical carcinoma growth. These data raise the possibility that cMET is a potential therapeutic target for adrenocortical carcinoma. In addition, the activation of HGF/cMET pathway was associated with increased cell proliferation and reduced apoptosis based on our immunohistochemical analysis of the tissue microarray. We also found that HGF promotes H295R cell growth in vitro. In other cancer models, HGF secretion was reported to be produced by tumor-derived fibroblasts and to have a paracrine role in stimulating tumor growth (28).

Serum HGF is elevated in different malignancies; it has prognostic value, is correlated with disease burden, and can be used to identify responders to systemic therapy in a variety of solid and hematologic malignancies (34–38). However, circulating HGF can be nonspecific and transiently elevated in other non-neoplastic disease processes (39–41). We identified HGF-induced cell growth in the H295R adrenocortical carcinoma cell line. A complex interaction exists between HGF/cMET pathway and other important signaling pathways. HGF stimulates tumor angiogenesis via enhancing endothelial cell proliferation and motility. These proangiogenic effects are mediated by increasing the production of angiogenic cytokines, such as VEGF and IL8, and by direct cMET activation (21, 22).

The molecular mechanisms responsible for cMET and HGF overexpression in adrenocortical carcinoma remain unclear and warrant investigation. Somatic mutations of MET are rare in adrenocortical carcinoma (42); however, the genome area
where MET is located is commonly amplified in adrenocortical carcinoma (15). We found somatic MET alteration in only one out of 14 adrenocortical carcinoma specimens and this opens the door for other mechanisms to be responsible for cMET activation such as gene amplification as reported in other solid malignancies (43). The adrenocortical carcinoma genomic atlas (adrenocortical carcinoma TCGA) data shows genomic amplification on MET in adrenocortical carcinoma patients compared with normal adrenal cortex. Thus, both MET amplification and transcriptional induction after exposure to radiation or chemotherapy are likely responsible for cMET overexpression.

In this study, we have identified activation of HGF/cMET signaling pathway as a driver of adrenocortical carcinoma tumorigenesis and at the same time a potential Achilles’ heel of this malignancy. In fact, by combining multiple functional omics screenings with tissue microarray analysis, in vitro assays, animal modeling, and pharmaceutical intervention, we demonstrated for the first time that HGF/cMET signaling pathway played a central role in adrenocortical carcinoma tumorigenesis, discovering a previously unknown therapeutic opportunity for clinical management of this aggressive malignancy. Therefore, emerging cMET inhibitors hold promise as a potential breakthrough in adrenocortical carcinoma treatment.

Moreover, the findings that adrenocortical carcinoma cells rapidly upregulated cMET expression as an emergency response to radiation and chemotherapy, and that MET upregulation was associated with enrichment of major genes responsible for anticancer therapy resistance, survival, and drug metabolism, could be of significant interest for the field of cancer therapy innovation. In fact, these findings may establish a scientific foundation for using cMET inhibitors to overcome drug resistance in adrenocortical carcinoma, which is currently a major challenge in adrenocortical carcinoma treatment (1, 4, 31, 32). Besides, whether the fast increase in MET expression after anticancer treatments is a particular feature of adrenocortical carcinoma cells or a common
Figure 7.

Increased HGF/cMET signaling is associated with enhanced proliferation, tumor growth, and reduced apoptosis in adrenocortical carcinoma. A, cell viability measured by MTT assay of NCI-H295R cells cultured at different concentrations of recombinant human HGF for 7 days. B, knockdown of MET expression by lentiviral shRNAs decreases adrenocortical carcinoma cell proliferation. C, knockdown of MET expression by lentiviral shRNAs decreases percentages of adrenocortical carcinoma cells in G2–M or S-phase. D, cell-cycle progression analysis showing the important role of cMET in adrenocortical carcinoma cell proliferation. E, mean tumor volume in mice at different weeks after xenografting of H295R–GFP–shRNA or H295R–cMET–shRNA cells (5 mice per group). F, mean tumor weights in mice 6 weeks after xenografting of H295R–GFP–shRNA or H295R–cMET–shRNA cells (5 mice per group; left) and representative images of xenografted tumors harvested from the mice (right; scale bars, 5 mm). G, mean volumes of tumors formed from xenografted H295R cells at different weeks after treatment of randomized control and cabozantinib-treated mice (6 mice per group). H, mean weights of tumors from randomized control and cabozantinib-treated mice after 6 weeks of treatment (6 mice per group; left) and representative images of xenografted tumors harvested from the mice (right; scale bars, 5 mm). Statistical significance of data in F and H was calculated by one-way ANOVA. The error bars represent 95% confidence intervals; ***, $P < 0.001$; ****, $P < 0.0001$. 

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phenomenon across many types of cancers remain to be explored. In addition, how adrenocortical carcinoma cells induce MET expression after anticancer therapies and what is the mechanism for crosstalk between MET and CYP450 family are interesting questions that warrant further study. We think that a complete understanding about the contribution of HGF/cMET signaling in adrenocortical carcinoma could perhaps establish activation of HGF/cMET pathway as a predictive marker for tumor progression and response to therapies, which may better stratification of adrenocortical carcinoma patients, optimize treatment plans, and ameliorate therapeutic outcomes.

In addition, despite remarkable progress in the field of adrenocortical carcinoma, the process and mechanisms of adrenocortical carcinoma evolution still remain largely unclear (30, 33, 44–47). It is also undetermined whether adrenocortical carcinoma evolutionary process originates from adrenal adenoma (30, 45, 47). There are several evidences supporting the adenoma–carcinoma sequential tumorigenesis but more direct proofs are needed to elucidate this mysterious evolution. Two studies performed by Bernard and colleagues and Trezzi and colleagues found the presence of malignant components within adrenal adenomas in occasional cases (44, 45). Moreover, Heaton and colleagues has recently developed a mouse model with enhanced IGF2 expression and increased β-catenin stability to temporarily promote adrenal cortical hyperplasia progression to the formation of adenomas and seldom carcinomas (46). In addition, high-resolution genomic analyses performed by Ronchi and colleagues pointed out several common molecular genetic signatures and some shared signaling transduction pathways (i.e., Notch and Wnt/β-catenin pathways) in adrenal adenomas and carcinomas (33), which suggests a possible common origin. In our study, we observed a seemingly gradual increase in HGF/cMET pathway upregulation and activation from normal adrenal cortex to adrenal adenoma and then carcinoma. Furthermore, when the HGF/cMET pathway is highly activated in adrenocortical carcinoma, their whole gene expression landscapes are reprogrammed toward promoting cancer hallmarks deregulation, accelerating tumorigenesis, enabling drug resistance while inhibiting tumor-suppressing activities. These findings suggest that HGF/cMET signaling activation could be a landmark in the evolutionary process and tumorigenesis sequence of adrenal cortical carcinoma.

The relatively small number of studied specimens in our report is a common shortcoming in studies seeking to find new signaling pathways in adrenocortical carcinoma, but highlights the need for collaborative work to establish the prognostic value of serum HGF as well as adrenocortical carcinoma cMET expression (48–50). The paucity of adrenocortical carcinoma cell lines is another limitation in adrenocortical carcinoma research. However, we share the view of other groups that the H295R cell line is the closest model to corticosteroid-producing adrenocortical carcinoma (51). Considering the heterogeneity of adrenocortical carcinoma, there is a need to validate our findings in a large cohort of patients via a multi-institutional collaboration, and to incorporate HGF measurements in future prospective studies to assess its role as a prognostic marker in adrenocortical carcinoma. Future work is also necessary to clarify the effect of cMET signaling on adrenocortical carcinoma resistance to currently used chemotherapy strategies in adrenocortical carcinoma as well as exploring the effect of HGF/cMET inhibition on adrenocortical carcinoma and determining the mechanisms involved in cMET upregulation in adrenocortical carcinoma. A better understanding of this relationship may provide a rationale for combination therapy.

In summary, we found that HGF/cMET are expressed at a higher level in adrenocortical carcinoma than in adrenal adenomas and normal cortex. We have also shown that adrenocortical carcinoma cells produce HGF, leading to positive autocrine feedback, which promotes adrenocortical carcinoma cell growth and proliferation. Moreover, activation of HGF/cMET appears to enhance adrenocortical carcinoma proliferation/growth, tumor-related angiogenesis, chemotherapy resistance, and cell survival. Furthermore, commonly used chemotherapeutic agents and radiation increased cMET expression in vitro and cMET inhibition reduced adrenocortical carcinoma growth in vitro and in vivo. Therefore, our data suggest that cMET may be a valuable therapeutic target for adrenocortical carcinoma, and further investigation of combinations of new cMET inhibitors alone or in combination with current therapies may lead to clinical breakthroughs in management of this disease. This study provides preliminary data about HGF/cMET activation as a possible predictive marker for adrenocortical carcinoma progression and response to therapies, which may improve stratification of adrenocortical carcinoma patients and clinical outcomes.

Disclosure of Potential Conflicts of Interest
S.-C.J. Yeung reports receiving a commercial research grant from DepoMed. No potential conflicts of interest were disclosed by the other authors.

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