Cancer Therapy: Preclinical

**Cabozantinib Suppresses Tumor Growth and Metastasis in Hepatocellular Carcinoma by a Dual Blockade of VEGFR2 and MET**

Qingfeng Xiang1, Weiqiang Chen4, Meng Ren2, Jingnan Wang3, Hongwu Zhang3, David Y.B. Deng3, Lei Zhang1, Changzhen Shang1, and Yajin Chen1

**Abstract**

**Purpose:** MET signaling has been suggested a potential role in hepatocellular carcinoma (HCC) and associated with prometastasis during antiangiogenesis therapy. We investigated the potential association between MET expression and therapeutic response to sorafenib in patients with HCC. Antitumor effects of cabozantinib, a dual inhibitor of MET and VEGFR2, were examined in cultured HCC cells as well as in vivo models.

**Experimental Design:** Total MET and phosphorylated MET (p-MET) were measured in 29 resected HCC specimens, and correlated with response to sorafenib as postoperative adjuvant therapy. In the second set of experiments using cultured HCC cells, and mouse xenograft and metastatic models, effects of cabozantinib were examined.

**Results:** High level of p-MET in resected HCC specimens was associated with resistance to adjuvant sorafenib therapy. In cultured HCC cells that expressed p-MET, cabozantinib inhibited the activity of MET and its downstream effectors, leading to G1-phase arrest. Cabozantinib inhibited tumor growth in p-MET–positive and p-MET–negative HCC by decreasing angiogenesis, inhibiting proliferation, and promoting apoptosis, but it exhibited more profound efficacy in p-MET–positive HCC xenografts. Cabozantinib blocked the hepatocyte growth factor (HGF)–stimulated MET pathway and inhibited the migration and invasion of the HCC cells. Notably, cabozantinib reduced the number of metastatic lesions in the lung and liver in the experimental metastatic mouse model.

**Conclusions:** Patients with HCC with high level of p-MET are associated with resistance to adjuvant sorafenib treatment. The dual blockade of VEGFR2 and MET by cabozantinib has significant antitumor activities in HCC, and the activation of MET in HCC may be a promising efficacy-predicting biomarker.

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**Introduction**

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer-related death worldwide (1). Despite improvements in diagnostic and therapeutic strategies, the prognosis of HCC still remains poor (2–4). Inhibiting angiogenesis has been used as a strategy in the treatment of HCC (5, 6). For example, sorafenib, a VEGF receptor (VEGFR) inhibitor with activity against platelet-derived growth factor receptor (PDGFR), c-Kit receptor, RAF and p38 signal transduction pathways, has become a standard treatment in patients with advanced HCC (7). Even though sorafenib improves the median survival in advanced HCC, the median overall survival remains less than 1 year partly due to that many patients eventually become resistant to this drug (8, 9). In addition, sorafenib, like other VEGFR inhibitors such as sunitinib and cediranib, possesses the possibility to increase the invasiveness and/or metastatic potential of tumors (10–12). Thus, developing inhibitors that simultaneously inhibit VEGF and other pathways involved in tumor invasion and metastasis may confer broad and potent antitumor efficacy.

MET, a transmembrane tyrosine kinase receptor for hepatocyte growth factor (HGF), has been observed to play an important role in the development of human cancers and drug resistance in cancer cells (13–15). Moreover,
overexpression of MET has been reported in various types of human cancers, including HCC (13, 14). Blocking MET expression by gene therapy reduces cell proliferation, colony formation, and migration in vitro and suppresses tumor growth, angiogenesis, and metastasis in vivo in multiple HCC cell lines (16–18). Interestingly, emerging evidence demonstrated that tumor vascular pruning caused by inhibition of the VEGF pathway led to the induction of hypoxia and subsequently triggered MET expression, which enhances tumor invasion and metastasis (10, 11, 19). Therefore, blockade of both MET and VEGFR pathways may achieve better treatment outcome in HCC.

Tivantinib was initially reported as a MET-selective inhibitor and demonstrated a nearly doubling of progression-free survival and overall survival in patients with HCC with high expression of MET (20). However, subsequent studies confirmed that tivantinib is not a MET inhibitor but an antimitotic agent that kills tumor cells independently of MET (21, 22). Cabozantinib is an oral small-molecule tyrosine kinase inhibitor that blocks phosphorylation of MET and VEGFR2 and also has activity against AXL, RET, and KIT (23). Results from phase II and III clinical trials demonstrated that cabozantinib exhibited encouraging clinical activity in multiple human cancers including castration-resistant prostate cancer, medullary thyroid cancer, and HCC with manageable side effects (24–26). Although cabozantinib has clinical benefit, the antitumor mechanism of cabozantinib in HCC has not been fully elucidated. Furthermore, antitumor agents that administered to patients before knowing their mechanism of action may be misleading in the development of predictive biomarkers. In the present study, we investigated whether cabozantinib could inhibit tumor growth and metastasis, and explored the molecular mechanism of antitumor activity of cabozantinib in HCC.

**Materials and Methods**

**Patients and specimens**

Archival HCC specimens were obtained from 29 patients who accepted potentially curative treatment of hepatic resection at Sun Yat-sen Memorial Hospital of Sun Yat-sen University (Guangzhou, China) between January 2008 and December 2012. These patients were pathologically diagnosed as HCC with microvascular invasion. Because microvascular invasion is one of the most powerful factors associated with the recurrence of HCC after resection (27), patients consented to take sorafenib as the adjuvant therapy. The criteria for following-up, definition of sorafenib resistance and high expression of MET were described in the Supplementary Materials and Methods. The characteristics of all patients were summarized in Supplementary Table S1.

**Cell lines and culture conditions**

SK-HEP1 and HepG2 cells were obtained from the American Type Culture Collection. MHCC97L and MHCC97H cells were supplied by the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). These cell lines were maintained in Dulbecco’s Modified Eagle Medium (DMEM; GIBCO BRL) supplemented with 10% FBS (GIBCO BRL), 100 U/mL penicillin, and 100 μg/mL streptomycin. HUVECs (human umbilical vein endothelial cells) were purchased from ScienCell Research Laboratories and were maintained in EBM-2 medium (Cambrex Bio Science Inc.) according to the manufacturer’s instructions. The four HCC cell lines were authenticated using short tandem repeat DNA testing by Beijing Micoread Gene Tech., Co., Ltd. in 2013. HUVECs were not authenticated by the authors. All cell cultures were maintained at 37°C in a CO2 incubator with a controlled humidified atmosphere composed of 95% air and 5% CO2.

**Reagents and antibodies**

Reagents and antibodies used in this study were described in the Supplementary Materials and Methods.

**Cell viability, colony formation, cell cycle, and apoptosis analyses**

Cell viability, colony formation, cell cycle, and apoptosis analyses were performed as described in the Supplementary Materials and Methods.

**Western blot analysis**

Cells or isolated independent tissues (lungs and tumors) from vehicle control- and cabozantinib-treated mice were lysed with RIPA Lysis Buffer (Santa Cruz Biotechnology) containing protease inhibitors (Complete; Roche) and phosphatase inhibitors (PhosStop; Roche). The protein concentration was determined using a bicinchoninic acid assay (Beyotime Bio Science Inc.) and equalized before loading. A total of 25 to 50 μg of protein were separated by SDS-PAGE and transferred onto polyvinylidene difluoride membranes. Membranes were blocked and blotted with the relevant antibodies. Horseradish peroxidase–conjugated secondary antibodies were detected with an enhanced...
chemiluminescence reagent (Millipore Corp.). GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used as a loading control. All antibody dilutions were 1:1,000 except for the GAPDH antibody, which was used at a dilution of 1:5,000.

**In vitro migration and invasion assays**

Wound-healing and Transwell assays were used to examine migration of HCC cells. The invasiveness of cells was determined as described in the Supplementary Materials and Methods.

**Animal experiments**

**Animal care.** Female BALB/c athymic nude mice, 5- to 6-week-old (Experimental Animal Center of Sun Yat-sen University, China), were used for *in vivo* studies. All animals were fed a standard diet *ad libitum* and housed in a temperature-controlled animal facility with a 12/12 hours light/dark cycle. All procedures were performed according to the NIH Guide for Care and Use of Laboratory Animals and were approved by the Bioethics Committee of Sun Yat-sen University.

**Tumor implantation and growth**

MHCC97H and HepG2 xenograft models were established by subcutaneous injection of tumor cells (5 × 10^7/mL) in PBS with Matrigel at a 1:1 ratio. The cell suspension was injected in a total volume of 0.2 mL into the right flank of the mice and was allowed to grow for 2 weeks to reach a tumor size of approximately 80 to 200 mm^3^. The mice were then randomized into three groups (n = 8/group): vehicle control (ddH_2O, orally), cabozantinib (10 mg/kg/d, orally), or cabozantinib (30 mg/kg/d, orally) for 14 consecutive days. Tumor dimensions and body weights were measured every 2 days starting with the first day of treatment. Tumor volume (mm^3^) was calculated by the following formula: \( V = \frac{4}{3} \pi r^3 \), where \( r \) refers to the larger and smaller dimensions collected at each measurement. The mice were sacrificed after 14 days of treatment. Solid tumors were excised, weighed, and their livers and lungs were weighed and sampled for tissue sectioning. To examine the metastases, 100 sequential sections (5 μm) were cut from the lungs and livers of each mouse, and every 10th section was stained with hematoxylin and eosin (H&E). Expression of phosphorylated MET (p-MET) in each group was determined by immunohistochemistry (IHC).

**Immunohistochemical analysis**

Frozen, 5-μm thick sections of tumor samples were prepared to determine vessel density with an anti-CD31 (1:100) antibody. To evaluate proliferation and apoptosis, 5-μm paraffin-embedded sections were stained with anti–Ki-67 (1:50) and anti-cleaved PARP (1:50) antibodies, respectively. After blocking endogenous peroxidase activity, the sections were incubated overnight with the primary antibodies at 4°C. Detection was completed with the Polink-2 Plus IHC Detection System (Beijing Zhongshan Biotechnology Co.) according to the manufacturer’s instructions. Sections were visualized by adding diamobenzidine (DAB kit; Beijing ZhongShan Biotechnology Co.) according to the manufacturer’s instructions. Sections were visualized by adding diamobenzidine (DAB kit; Beijing ZhongShan Biotechnology Co.) according to the manufacturer’s instructions. Negative controls were obtained by omitting the primary antibody. Staining was evaluated by two independent observers. To quantify the mean vessel density (MVD) in sections stained for CD31, 10 random fields per tumor sample at ×200 magnification were captured and quantified as CD31-positive area/total area by Image-Pro Plus software (Media Cybernetics, Inc.). For Ki-67 and cleaved PARP, only nuclear immunoreactivity was considered positive. The proliferation index and apoptosis index corresponded to the number of labeled Ki-67 or cleaved PARP cells among at least 500 cells per region and are expressed as percentages.

**Statistical analysis**

Statistical analyses were performed with mean ± SD values using the Student *t* test and two-way ANOVA with the Bonferroni correction. Statistical significance was concluded at *P* < 0.05.

**Results**

**The expression of p-MET in resected HCC specimens is associated with resistance to adjuvant sorafenib therapy**

The expression of MET in HCC specimens was detected by immunostaining. We observed that MET is overexpressed in most of tumor tissues (data not shown), whereas p-MET is highly expressed in approximately 27.6% patients with HCC. Notably, we found that positive p-MET staining was associated with the therapeutic response to sorafenib. Specifically, seven of 12 (58.3%) cases with sorafenib resistance have high p-MET expression, whereas only one of 17 (5.88%) sorafenib-sensitive cases were observed to have positive staining for p-MET (Supplementary Fig. S1).
The effect of cabozantinib on proliferation in each HCC cell line is shown in Fig. 1. Cabozantinib inhibited cell growth in a concentration-dependent manner in MHCC97L and MHCC97H cells, with IC₅₀ values of 13.47 and 9.466 nmol/L, respectively. SK-HEP1 and HepG2 cells were much less sensitive to cabozantinib (IC₅₀ = 4.306 and 5.040 nmol/L, respectively). Similar results were obtained from the colony formation assay (Supplementary Fig. S2). To analyze the mechanisms by which cabozantinib inhibited cell proliferation, flow cytometric analysis was conducted to analyze the cell cycle and apoptosis of cells after treatment with various concentrations of cabozantinib. As shown in Fig. 2A, in both MHCC97L and MHCC97H cells, cabozantinib markedly increased the percentage of cells in the G1-phase, whereas decreasing the percentage of cells in the S-phase. Cyclin D1 is a critical regulator of the G1–S transition (29). Upregulation of cyclin D1 results in rapid growth of a subset of HCC (30). Western blot analysis indicated that cyclin D1 expression in MHCC97L and MHCC97H cells was reduced after treatment with cabozantinib for 24 hours (Supplementary Fig. S3A). Notably, after treatment with cabozantinib, SK-HEP1 and HepG2 cells showed a decrease in the G1-phase and an increase in the G2-phase (Fig. 2B). Cabozantinib induced apoptosis in SK-HEP1 and HepG2 cells, but not in MHCC97L and MHCC97H cells (Fig. 2C and D and Supplementary Fig. S3B). The data presented above collectively suggest that different mechanisms seem to be involved in the antiproliferative effect of cabozantinib on MHCC97L, MHCC97H, SK-HEP1, and HepG2 cells.

**Cabozantinib inhibits MET and VEGFR2 phosphorylation and their downstream effectors in vitro**

Western blot analysis demonstrated that cabozantinib-sensitive MHCC97L and MHCC97H cells displayed a dramatic elevation in MET phosphorylation, compared with SK-HEP1 and HepG2 cells. No VEGFR2 expression was detected in cultured HCC cells (Fig. 3A), which indicates that the inhibitory activity of cabozantinib on VEGFR2 is not involved in its antiproliferative effects on these HCC cells in vitro.

Next, we investigated the effect of cabozantinib treatment on MET-dependent signaling pathways. Marked suppression of p-MET was observed in MHCC97L and MHCC97H cells tested after 4 hours incubation with cabozantinib at concentrations as low as 10 to 100 nmol/L. Moreover, treatment with these doses also effectively abrogated the phosphorylation of downstream effectors, such as STAT3, AKT, and ERK1/2 (Fig. 3B). Thus, constitutive activation of these proliferative and survival effectors in MHCC97L and MHCC97H cells seems to depend specifically on MET signaling. In contrast, in SK-HEP1 and HepG2 cells, in which MET is not constitutively phosphorylated, cabozantinib at a dose of 100 nmol/L had no demonstrable effect on the phosphorylation of STAT3, AKT, or ERK1/2, indicating that these proliferative and survival effectors are likely activated through alternative growth factor

![Figure 1. Cabozantinib inhibits HCC cell proliferation. MHCC97L, MHCC97H, SK-HEP1, and HepG2 cells were treated with different concentrations of cabozantinib for 72 hours in DMEM containing 10% FBS. Each point, the mean ± SD for three independent experiments. IC₅₀ was calculated by nonlinear regression analysis using GraphPad Prism software.](image-url)
Figure 2. Effects of cabozantinib on cell-cycle progression and apoptosis in HCC cells. A and B, effect of cabozantinib on the cell cycle. MHCC97L, MHCC97H, SK-HEP1, and HepG2 cells were treated with either 0.1% DMSO (dimethyl sulfoxide) or cabozantinib for 24 hours. After treatment, cells were harvested, fixed, and stained with propidium iodide (PI) for flow cytometric analysis. Data were analyzed using ModFit and were reported as the mean ± SD. C and D, apoptosis of HCC cells detected by the Annexin V–FITC/propidium iodide–binding assay. Cells were treated with either 0.1% DMSO or cabozantinib for 48 hours at the indicated concentrations, and then stained with Annexin V–FITC and propidium iodide. The rate of apoptosis was determined using a flow cytometer, and data were analyzed using Kaluza software and were reported as the mean ± SD. The results are representative of three independent experiments. *, P < 0.05 and ***, P < 0.01, versus control.
receptors. An increase in the concentration of cabozantinib, up to 5,000 and 10,000 nmol/L, had a significant impact on the phosphorylation of STAT3, AKT, and ERK1/2 in both SK-HEP1 and HepG2 cells (Fig. 3B). This effect may be because cabozantinib has a nonspecific inhibitory effect on these effectors or exerted through the inhibition of other cancer-specific cabozantinib targets, such as AXL, RET, and KIT.

In a cytokine-stimulated tyrosine kinase activity assay, we found that cabozantinib treatment resulted in the marked inhibition of cytokine-stimulated phosphorylation of MET and VEGFR2 and their resultant downstream effectors in HUVECs (Fig. 3C). In a concentration-dependent manner, cabozantinib eliminated HGF-induced MET phosphorylation and its downstream effectors STAT3, Akt, and Erk-1/2 in both SK-HEP1 and HepG2 cells (Fig. 3D).

**Cabozantinib inhibits HGF-induced cell motility and invasion**

Because MET is not necessary for proliferation of SK-HEP1 and HepG2 cells, we used these two cell lines to test whether cabozantinib has an effect on motility and invasion. We observed that HGF enhanced the migration and invasion of SK-HEP1 and HepG2 cells as evaluated by a wound-healing and Transwell assay. Moreover, at a concentration that has minimal impact on growth, cabozantinib inhibited HGF-induced migration and invasion in both SK-HEP1 and HepG2 cells (Fig. 4). These findings reflect the potential antimetastatic effect of cabozantinib in HCC cells.

**In vivo efficacy and mechanism of cabozantinib against MHCC97H and HepG2 xenografts**

To examined that cabozantinib inhibits VEGFR2 and MET signaling activity in vivo, established MHCC97H xenografts (n = 3/group) were treated daily with an oral dose of vehicle or cabozantinib at 30 mg/kg for 3 days. As shown in Supplementary Fig. S4, administration of cabozantinib resulted in significant inhibition of VEGFR2 and MET phosphorylation in mice lungs and tumors, respectively, compared with the vehicle-treated control group. Moreover, the inhibition of downstream MET effectors, such as STAT3, AKT, and ERK1/2, was also detected in MHCC97H tumors.
The results in Supplementary Table S2 and Fig. 5A demonstrate that cabozantinib at concentrations of both 10 and 30 mg/kg displayed a good anticancer effect on MHCC97H xenografts; their tumor growth inhibition (TGI) rates were 53.4% and 84.6%, respectively. Treatment of HepG2 xenografts with an identical treatment scheme led to 27.5% and 59.1% TGI, compared with the vehicle-treated control group (Supplementary Table S2; Fig. 5B). These differences in efficacy imply that the overexpression of p-MET may identify a sensitivity index for cabozantinib treatment in HCC.

We next evaluated the antiangiogenic, antiproliferative, and proapoptotic effects of cabozantinib in treated tumor xenografts. Immunohistochemical analyses revealed that cabozantinib decreased the MVD in MHCC97H xenografts by 49.9% and 90% at doses of 10 and 30 mg/kg, respectively, compared with the vehicle-treated control (Fig. 6). MVD in HepG2 xenografts was reduced by 58.3% and 87.1% at doses of 10 and 30 mg/kg, respectively, compared with the vehicle-treated control. In addition, MHCC97H and HepG2 xenografts treated with cabozantinib revealed a significant reduction in proliferation (the percentage of Ki-67–positive cells) and an increase in apoptosis (the percentage of cleaved PARP-positive cells).

Cabozahtinib prevents metastasis of SK-HEP1 cells to the lung and liver

To determine whether cabozantinib treatment could reduce metastasis, SK-HEP1 cells were directly injected into the tail vein of female nude mice. After injection, mice received sorafenib or cabozantinib treatment for 4 weeks. We found that the formation of metastases in the lung and liver
liver was reduced by 53.7% and 52.9% in the cabozantinib-treated group, respectively, compared with the vehicle-treated group (Fig. 7). Interestingly, lungs and livers from mice in the sorafenib-treated group displayed an apparent increase in metastatic foci. Additional evidence of the inhibition of metastasis in the cabozantinib-treated group was confirmed by the significant difference in whole-lung wet weights among control, sorafenib, and cabozantinib treatment groups (Fig. 7A). Comparison of p-MET, as assessed by IHC, in mice treated with ddH2O or sorafenib corroborated the known positive relationship between VEGF signaling inhibition and MET activation (31–33). After sorafenib treatment for 28 days, staining for p-MET was strong and widespread in lung tissue and metastatic foci in the liver. Staining for p-MET was weak or absent in the cabozantinib-treated group (Supplementary Fig. S5). Cabozantinib treatment was well tolerated, as determined by stable body weights throughout the 28 days treatment period.

Discussion

Targeting angiogenesis has become an established therapeutic approach to fighting solid tumor growth in patients with cancer, and the systemic therapy with sorafenib represents a milestone in advanced HCC. However, the benefit of sorafenib in clinical therapy is marginal and transient (8, 9). The MET pathway has been found involved in gefitinib resistance in lung cancer (15). In line with this, we revealed that high level of activated MET in HCC is associated with resistance to adjuvant sorafenib treatment. Moreover, we demonstrated that cabozantinib, a dual inhibitor of MET and VEGFR2, could inhibit the growth, migration, invasion, and metastasis of HCC both in vitro and in vivo.

In tumor cell growth inhibition assays, cabozantinib could inhibit the growth of p-MET expressing cells (MHCC97L and MHCC97H) at low concentrations, but required much higher concentration in p-MET-negative cells (SK-HEP1 and HepG2). Flow cytometry analysis revealed that cabozantinib suppressed the proliferation of MHCC97L and MHCC97H cells by causing G1-phase cell-cycle arrest without inducing apoptosis. The expression of cyclin D1, which is a critical regulator of the G1-S transition, was markedly blocked by cabozantinib. These results are supported by the study of Zhang and colleagues, who showed that MET knockout induces significant G1 arrest and a decrease of cyclin D1 in MHCC97L and MCC97H cells (16). Cabozantinib displayed a significant concentration-related antiproliferative effect on MHCC97L and MHCC97H cells at approximately 10 nmol/L, which was accompanied by a reduction of phosphorylation of MET and its downstream effectors STAT3, Akt, and Erk1/2. These findings highlight that cabozantinib suppressed the proliferation of MHCC97L and MHCC97H cells by impeding the MET pathway.

Consistent with the in vitro study, cabozantinib showed an anti-MET and -VEGFR2 pathway activity in the MHCC97H xenografts model. The treatment of MHCC97H xenografts with cabozantinib resulted in a more pronounced TGI compared with its efficacy on HepG2 xenografts, suggesting that p-MET amplification may be a molecular marker of susceptibility to cabozantinib treatment in HCC cells. Both MHCC97H and HepG2 xenografts treated with cabozantinib showed reduced microvessel density, suppressed proliferation, and increased apoptosis. On the basis of our findings, we propose that the antitumor effect of cabozantinib on p-MET-positive MHCC97H xenografts seems to be mediated by inhibiting tumor angiogenesis (anti-VEGFR2 effect) and by directly inhibiting tumor cell proliferation (anti-MET effect). For p-MET-negative HepG2 xenografts, impeding stromal angiogenesis through VEGFR2 inhibition may contribute to the dominant abrogation of tumor growth. Because patients with HCC may have MET-negative disease (34), we propose that MET activation may be a useful biomarker in cabozantinib clinical trials in identifying patients with HCC with potential for the greatest benefit, by predicting durable tumor shrinkage and increased tumor response rate. However, the degree to which inhibition of the MET signaling pathway contributes to antiangiogenesis and inhibition of other cabozantinib pathways requires further investigation.

Figure 5. Cabozantinib abrogates tumor growth in the xenograft mouse model. A and B, MHCC97H and HepG2 cells were subcutaneously implanted in nude mice as described in Materials and Methods. Mice bearing tumors xenografts were treated daily with vehicle or either 10 or 30 mg/kg of cabozantinib for 14 days. The image shows the tumor size of MHCC97H and HepG2 xenografts at the end of the experiment. Data, the mean ± SD of the tumor volume and tumor weight for each group of eight experimental animals. *, P < 0.05; **, P < 0.01; the treated group versus the control group.
targets contributes to tumor growth inhibition in vivo remains to be clarified.

It has been reported that cabozantinib suppressed cell migration and invasion in various types of tumor cells (23, 35). Consistently, our results showed that cabozantinib inhibited HGF-stimulated migration and invasion in HCC cell lines. Notably, treatment of SK-HEP1 and HepG2 cells with low concentration of cabozantinib had minimal impact on growth but strongly reduced migration and invasion potential, suggesting a role of cabozantinib on cell motility and invasion without affecting proliferation.

Recent studies have demonstrated that antiangiogenesis agents could increase local invasion and distant metastasis during or after treatment (11, 12, 36, 37). For example, VEGFR inhibitors, such as sorafenib and sunitinib, can result in upregulation of MET, leading to promotion of metastasis (19, 32, 38). In support of this, we observed that sorafenib treatment promoted metastasis in the lung and liver, and accompany with activation of MET in experimental metastasis models with SK-HEP1 cells. This finding argues that simultaneously targeting MET and VEGFR2 may circumvent the "metastatic escape pathways." Indeed, mice treated with cabozantinib had fewer metastatic foci in lung and liver tissues compared with control- and sorafenib-treated groups, suggesting that cabozantinib could reduce tumor metastasis mainly through inactivation of MET. Interestingly, our observations raise the possibility that MET could be activated during tumor progression and sorafenib treatment.
leading to enhanced metastasis, thereby identifying a potential target for therapeutic intervention.

In summary, our study revealed that the high level of p-MET in HCC tissue could be a prognosticator of resistance to adjuvant sorafenib therapy. The inhibition of both VEGFR2 and MET signaling pathways by cabozantinib could have considerable therapeutic effects in HCC in vitro and in vivo. Also, the presence of MET activation in HCC may be a promising biomarker for predicting the response to cabozantinib treatment. Altogether, cabozantinib could be a useful agent for inhibiting tumor growth, angiogenesis, and metastasis in HCC with dysregulated MET and VEGFR2 signaling pathways.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.
Antitumor Activity of Cabozantinib in HCC

Authors’ Contributions
Conception and design: M. Ren, Y. Chen, C. Shang
Development of methodology: Q. Xiang, Y. Chen
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Q. Xiang, J. Wang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Q. Xiang, W. Chen, J. Wang, H. Zhang
Writing, review, and/or revision of the manuscript: Q. Xiang, W. Chen, M. Ren, J. Wang, L. Zhang, Y. Chen
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): W. Chen, J. Wang, L. Zhang, Y. Chen, C. Shang
Study supervision: W. Chen, D.Y.B. Deng, Y. Chen

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Correction: Cabozantinib Suppresses Tumor Growth and Metastasis in Hepatocellular Carcinoma by a Dual Blockade of VEGFR2 and MET

In this article (Clin Cancer Res 2014;20:2959–70), which was published in the June 1, 2014, issue of Clinical Cancer Research (1), a reader alerted us to potential image manipulation in the control ×200 and sorafenib (30 mg/kg) ×200 panels in Fig. 7A. AACR Publications staff members and editors reviewed the figure and agreed that sections of the panels appeared to be identical. The authors admitted that an image of the sorafenib (30 mg/kg) ×200 panel was mistakenly included in the control ×200 panel with their submission. The authors have provided the correct version of the control ×200 panel for Fig. 7A below. The results and conclusions put forth in this article remain unchanged. The authors regret these errors.

Reference
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