Preclinical Development

Dovitinib Induces Apoptosis and Overcomes Sorafenib Resistance in Hepatocellular Carcinoma through SHP-1–Mediated Inhibition of STAT3

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Abstract

The multiple kinase inhibitor dovitinib is currently under clinical investigation for hepatocellular carcinoma (HCC). Here, we investigated the mechanistic basis for the effects of dovitinib in HCCs. Dovitinib showed significant antitumor activity in HCC cell lines PLC5, Hep3B, Sk-Hep1, and Huh-7. Dovitinib downregulated phospho-STAT3 (p-STAT3) at tyrosine 705 and subsequently reduced the levels of expression of STAT3-related proteins Mcl-1, survivin, and cyclin D1 in a time-dependent manner. Ectopic expression of STAT3 abolished the apoptotic effect of dovitinib, indicating that STAT3 is indispensable in mediating the effect of dovitinib in HCC. SHP-1 inhibitor reversed downregulation of p-STAT3 and apoptosis induced by dovitinib, and silencing of SHP-1 by RNA interference abolished the effects of dovitinib on p-STAT3, indicating that SHP-1, a protein tyrosine phosphatase, mediates the effects of dovitinib. Notably, dovitinib increased SHP-1 activity in HCC cells. Incubation of dovitinib with pure SHP-1 protein enhanced its phosphatase activity, indicating that dovitinib upregulates the activity of SHP-1 via direct interactions. In addition, dovitinib induced apoptosis in two sorafenib-resistant cell lines through inhibition of STAT3, and sorafenib-resistant cells showed significant activation of STAT3, suggesting that targeting STAT3 may be a useful approach to overcome drug resistance in HCC. Finally, in vivo, dovitinib significantly suppressed growth of both Huh-7 and PLC5 xenograft tumors and downregulated p-STAT3 by increasing SHP-1 activity. In conclusion, dovitinib induces significant apoptosis in HCC cells and sorafenib-resistant cells via SHP-1–mediated inhibition of STAT3. Mol Cancer Ther; 11(2); 1–12. ©2011 AACR.

Introduction

Human hepatocellular carcinoma (HCC) is one of the most prevalent solid tumors worldwide. Traditional systemic chemotherapy does not provide survival benefits in patients with HCC. Recently, sorafenib, an inhibitor of multiple kinases including Raf-1 and VEGF receptor (VEGFR), has shown benefits in patients with advanced HCC and was approved for use in HCC by the U.S. Food and Drug Administration in 2007 (1–3). Sorafenib is the only drug approved for HCC treatment; however, it only provides a modest effect, prolonging survival in patients with HCC from a median 7.9 to 10.7 months. Therefore, more effective new drugs are still urgently needed for HCC.

At the beginning of 2011, an open-label, randomized, phase II study of dovitinib (TKI258) in HCC was initiated in the Asia-Pacific region (NCT01232296). The purpose of this trial is to compare the safety and efficacy of dovitinib with sorafenib as a first-line treatment in adult patients with advanced HCC. Dovitinib is a small-molecule inhibitor of multiple receptor tyrosine kinases (RTK), including fibroblast growth factor receptor (FGFR), VEGFR, c-KIT, and FMS-like tyrosine kinase 3 (FLT3; ref. 4). According to previous studies, dovitinib exhibits potent antitumor activity in a broad range of preclinical animal models. For example, dovitinib induced apoptosis in FGFR-expressing mammary cells via inhibition of PI3K/Akt signaling pathway (5). In addition, dovitinib specifically inhibited proliferation and survival of primary cells and cell lines with FGFR1 fusion genes associated with the 8p11 myeloproliferative syndrome (6). Evidence of anti-tumor activity in melanoma and gastrointestinal stromal tumors warrants further investigation and other phase I studies are ongoing (7).

Recently, we reported that STAT3 plays a role in mediating the effect of sorafenib on HCC (8, 9). STAT3 plays a...
vital role in liver inflammation and tumor progression triggered by cytokines and growth factors such as epidermal growth factor receptor (EGFR), FGFR, and platelet-derived growth factor receptor (PDGFR) through tyrosine phosphorylation. In addition, STAT3-mediated downstream proteins, such as Mcl-1, cyclin D/E, survivin, bcl-2, and bcl-xl, are also associated with survival signaling (10). Furthermore, STAT3 is activated constitutively in numerous cancer cells and inhibits the expression of mediators necessary for immune activation against carcinogenesis (11, 12). Consequently, STAT3 has emerged as a promising target for cancer therapy.

Many protein families act as negative regulators of the STAT3 signaling pathway. SOCS family proteins, which have 2 domains, an Src homology 2 (SH2) domain and a C-terminal SOCS box, mediate the direct interaction with Janus—activated kinase (JAK) and repress the JAK/STAT pathway (13). In addition, JAK/STAT3 signaling is inactivated by various protein tyrosine phosphatases (PTP) such as SH2-domain-containing cytosolic phosphatases, SHP-1 and SHP-2 (14, 15). SHP-1 belongs to a family of non-receptor PTPs and consists of 2 SH2 domains that bind phosphorylated, a catalytic PTP domain and a C-terminal tail. Although many reports have investigated SHP-1 in hematopoietic cells, comparatively few reports have looked at the biologic importance of SHP-1 in solid tumors even though early studies have shown that SHP-1 is a potential tumor suppressor modulated in cancer progression (16, 17).

Here, we report that dovitinib directly and strongly induces SHP-1 activity that is associated with downregulation of p-STAT3 and growth inhibition of HCC cells. To better understand the molecular mechanism of dovitinib in HCC targeted therapy, we investigated the molecular events altered by dovitinib treatment in vitro and in vivo. The role of SHP-1 activity—mediated downregulation of p-STAT3 was confirmed, thus providing novel mechanistic insight into this molecular target for HCC. Moreover, we showed that dovitinib sensitizes sorafenib-resistant cells reinforcing the potential usefulness of dovitinib in the clinic.

Materials and Methods

Reagents and antibodies

Dovitinib (TKI258) was kindly provided by Novartis Pharmaceuticals. SHP-1 inhibitor was purchased from Cayman Chemical. Antibodies for immunoblotting such as cyclin D1 and PARP were purchased from Santa Cruz Biotechnology. Other antibodies such as survivin, phospho-STAT3 (Tyr705), STAT3, SHP-1, SHP-2, survivin, and PTP-1B were from Cell Signaling.

Cell culture

The Huh-7 HCC cell line was obtained from the Health Science Research Resources Bank (HSRRB; Osaka, Japan; JCRB0403). The PLC/PRF/5 (PLC5), Sk-Hep-1, and Hep3B cell lines were obtained from American Type Culture Collection (ATCC). All cells obtained from HSRRB or ATCC were immediately expanded and frozen down such that all cell lines could be restarted every 3 months from a frozen vial of the same batch of cells. No further authentication was done in our laboratory. Cells were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% FBS, 100 units/mL penicillin G, 100 μg/mL streptomycin sulfate, and 25 μg/mL amphotericin B in a humidified incubator at 37°C in an atmosphere of 5% CO2 in air. Primary cancer cells from consenting patients were also analyzed. Study protocols were approved by Institutional Review Board of the institution, and informed consent was obtained in accordance with the Declaration of Helsinki. Primary leukemia cells were collected from bone marrow (with blasts >80%) from patients with acute myelogenous leukemia. Cells were frozen in medium containing 10% dimethyl sulfoxide (DMSO) within 24 hours of harvesting. Human HCC samples were obtained from the patient who underwent tumor resection. The cells were isolated by mechanical mincing and digestion by collagenase.

Apoptosis analysis

The following 3 methods were used to assess drug-induced apoptotic cell death: measurement of apoptotic cells by flow cytometry (sub-G1), cell death detection ELISA for cytoplasmic histone-associated DNA fragments (Roche), and Western blot analysis for PARP and caspases cleavage. For measurement of sub-G1 percentage, HCC cells were treated with DMSO or dovitinib at the indicated dose for 24 hours. The percentage of apoptotic cells was shown by cell-cycle distribution using flow cytometry. Also, the specific detection of oligonucleosomes released into cytoplasm was quantified by cell death ELISA.

Gene knockdown using short interfering RNA

Smart-pool short interfering RNAs (siRNA), including control (D-001810-10), SHP-1, SHP-2, and PTP-1B were all purchased from Dharmacon Inc. The knockdown procedure was as described previously (18). Briefly, PLC5 cells were transfected with siRNAs against the phosphatases given above or the control sequence for 24 hours and then treated with dovitinib at the indicated concentrations. The cell extracts were analyzed by immunoblotting and propidium iodide staining.

PLC5 and Sk-Hep1 cells with ectopic expression of STAT3

STAT3 cDNA (KIAA1524) was purchased from Addgene plasmid repository (http://www.addgene.org/). PLC5 and Sk-Hep1 cells with ectopic expression of STAT3 derived from a single stable clone were prepared for evaluating the major target of dovitinib. Briefly, following transfection, cells were cultured in the presence of G418 (0.8 mg/mL) according to previous reports (19, 20). After 8 weeks of selection, surviving colonies, that is, those
arising from stably transfected cells were selected and individually amplified.

**SHP-1 phosphatase activity**
A RediPlate 96 EnzChek Tyrosine Phosphatase Assay kit (R-22067) was used for SHP-1 activity assay (Molecular Probes). The method was as described previously (8).

**STAT3 reporter assay**
PLC5 cells were seeded in a 96-well plate and pretreated with interleukin (IL)-6 at the dose 10 ng/μL for 30 minutes. The STAT3 Reporter Kit was purchased from SABiosciences.

**Determination of synergism**
Drug synergism was determined using the method of Chou and Talalay and the software package CalcuSyn (Biosoft). A combination index (CI) less than 1 was defined as synergism (19).

**Xenograft tumor growth**
Male NCr athymic nude mice (5–7 weeks of age) were obtained from the National Laboratory Animal Center (Taipei, Taiwan). All experimental procedures using these mice were carried out in accordance with protocols approved by National Taiwan University (Taipei, Taiwan). When Huh-7 or PLC5 tumors reached 100 mm³, mice received dovitinib (15 or 30 mg/kg) per os once daily. Controls received vehicle.

**Statistical analysis**
Comparisons of mean values were conducted using the independent samples t test in SPSS for Windows 11.5 software (SPSS, Inc.).

**Results**

**Multiple kinase inhibitor dovitinib shows antitumor effects in HCC cells**
To investigate the anticancer effects of dovitinib (Fig. 1A), we first assessed growth inhibition in response to dovitinib treatment in a panel of 4 human HCC cell lines: PLC5, Hep3B, SK-Hep1, and Huh-7. Cell viability was determined by MTT assay after treatment for 72 hours (up to 15 μmol/L). As shown in Fig. 1B, dovitinib significantly reduced cell viability at clinically relevant concentrations in a dose-dependent manner in all 4 cell lines. Next, we examined the apoptotic effect of dovitinib on HCC. Apoptotic cells (sub-G₀) were determined by flow cytometry. All 4 HCC cell lines were exposed to dovitinib at 3 different concentrations. The data shown in Fig. 1C indicate that dovitinib substantially increased apoptotic cell death in a dose-dependent manner (starting at 5 μmol/L). Dovitinib showed similar effects on apoptosis in all tested cell lines. We further confirmed the apoptotic effect of dovitinib by DNA fragmentation ELISA assay. Dovitinib caused dose-dependent DNA fragmentation in 4 HCC cell lines starting at a concentration of 2.5 μmol/L (Fig. 1D). These data indicate that dovitinib has considerable effects on growth inhibition and apoptosis in HCC cells.

**Downregulation of p-STAT3 contributes the apoptotic effect of dovitinib in HCC**
To elucidate the underlying mechanism by which dovitinib induces apoptosis in HCC, we examined the alterations in signal transduction induced by dovitinib in HCC. As shown in Fig. 2A, dovitinib downregulated phospho-STAT3 (p-STAT3) at tyrosine 705 in a dose-dependent manner (Fig. 2A). Notably, total STAT3 protein was not affected by dovitinib in a dose-escalation assay. The phosphorylation of STAT3 at tyrosine residue 705 responded to transactivation of STAT3 and triggered subsequent activation of proteins participating in cell survival and proliferation. Consequently, the downstream effectors driven by STAT3, such as Mcl-1, cyclin D1, and survivin, were also repressed by dovitinib in 4 HCC cell lines (Fig. 2A). Induction of apoptosis was further confirmed by the activation of caspase-9 and PARP cleavage in dovitinib-treated cells (Fig. 2A). Moreover, dovitinib downregulated p-STAT3 and Mcl-1 in association with PARP cleavage in a time-dependent manner in Sk-Hep1 and Huh-7 cells (Fig. 2B, left). Next, we used an ELISA assay to further examine the effect of dovitinib on p-STAT3. As shown in Fig. 2B, right, dovitinib significantly reduced p-STAT3 in PLC5 and Sk-Hep1 cells (P < 0.05). To further confirm that dovitinib decreased STAT3-driven transcription activity, we conducted a STAT3 reporter assay. As shown in Fig. 2C, dovitinib significantly decreased the transcription activity of STAT3 in a dose-escalation manner. In this assay, firefly luciferase reporter gene was constructed with a promoter region containing the STAT3-specific binding sites. Renilla luciferase reporter gene was used for quantification. Dovitinib abolished nearly 50% of luciferase activity at the highest treatment dose. These results suggest that dovitinib may inhibit Mcl-1, cyclin D1, and survivin via transcriptional repression of STAT3. To further validate the role of STAT3 in dovitinib-induced apoptosis in HCC, we next generated HCC cells with stable expression of STAT3. As shown in Fig. 2D, ectopic expression of STAT3 reversed downregulation of p-STAT3 and reduced the apoptotic effect of dovitinib in both PLC5 and SK-Hep1 cells, suggesting that STAT3 mediates dovitinib-induced apoptosis in HCC. Notably, overexpression of STAT3 did not suppress dovitinib-induced apoptosis completely, indicating that STAT3 might not be the only modulator to mediate the effect of dovitinib in HCC.

**SHP-1 plays a critical role in dovitinib-induced apoptosis and inhibition of p-STAT3**
Next, we attempted to elucidate the mechanism by which dovitinib downregulates p-STAT3 in HCC cells. We thus further examined the role of several protein phosphatases in dovitinib-induced inhibition of STAT3 signals. Several phosphatases have been reported to be...
associated with dephosphorylation of STAT3, including SHP-1, SHP-2, and PTP-1B (14, 17). First, we assessed whether SHP-1 phosphatase was associated with dovitinib-induced STAT3 inhibition. A specific SHP-1 inhibitor significantly reduced dovitinib-induced downregulation of p-STAT3 and apoptosis (Fig. 3A). In addition, silencing SHP-1 with siRNA protected cells from dovitinib-induced apoptosis (Fig. 3B). Importantly, downregulation of SHP1 by siRNA also abolished the effects of dovitinib on p-STAT3. On the other hand, silencing either SHP-2 or PTP-1B did not affect the effect of dovitinib on apoptosis and p-STAT3 in HCC (Fig. 3D). These data indicate that SHP-1 is indispensable in mediating the effect of dovitinib on p-STAT3 and apoptosis in HCC.

Dovitinib increases the phosphatase activity of SHP-1

To understand the effect of dovitinib on SHP-1, we further examined the phosphatase activity of SHP-1 in dovitinib-treated HCC cells. PLC5 and Sk-Hep1 cells were exposed to dovitinib at 10 μmol/L for 24 hours and then cell lysates were collected for detection of SHP-1 activity. Dovitinib increased SHP-1 activity significantly in both cell lines (Fig. 4A, left). To examine whether dovitinib

Figure 1. Multiple kinase inhibitor dovitinib shows antitumor effects in HCC cell lines. A, chemical structure of dovitinib. B, dose-escalation effects of dovitinib on cell viability in 4 HCC cell lines. Cells were exposed to dovitinib at the indicated doses for 72 hours and cell viability was assessed by MTT assay. Points, mean; bars, SD (n = 8). C, effects of dovitinib on apoptosis in HCC cells. Cells were exposed to dovitinib at the indicated doses for 24 hours and apoptotic cells were determined by flow cytometry (sub-G). D, effects of dovitinib on DNA fragmentation. Cells were exposed to dovitinib at the indicated concentrations for 24 hours. DNA fragmentation was measured by cell death detection ELISA. Points, mean; bars, SD (n = 6).
enhanced the activity of SHP-1 through direct interaction, we further tested the effect of dovitinib on SHP-1–containing cell lysates. Briefly, PLC5 cells were immunoprecipitated with anti-SHP-1 antibody. Protein extract that included SHP-1 complex was further incubated with dovitinib at 10 nmol/L for 30 minutes and then SHP-1 phosphatase activity assay was conducted. Our data showed that dovitinib did increase the phosphatase activity of SHP-1–containing lysates (Fig. 4A, middle). For more specific identification, we incubated pure SHP-1 recombinant protein with dovitinib at 10 nmol/L for 30 minutes and then assayed its phosphatase activity. SHP-1 phosphatase activity was increased up to approximately 5-fold with dovitinib treatment (Fig. 4A, right).
together, these data imply that dovitinib may interact with SHP-1 directly and increase its phosphatase activity. Moreover, 2 phosphorylation sites, Tyr536 and Ser591, have been reported to increase and decrease phosphatase SHP-1 activity, respectively (15, 21). We next examined the phosphorylation of SHP-1 in dovitinib-treated cells. As shown in Fig. 4B, the phosphorylation level of SHP-1 was not affected by dose-dependent treatment with dovitinib. Therefore, apparently dovitinib did not affect SHP-1 activity through altering the phosphorylation of SHP-1. In addition, we generated PLC5 cells with ectopic expression of SHP-1 to analyze the effect of dovitinib on SHP-1. We observed that dovitinib induced more apoptosis and inhibition of STAT3 in SHP-1-overexpressed cells (Fig. 4C). Notably, overexpression of SHP-1 downregulated p-STAT3. Collectively, these data suggest that dovitinib increases SHP-1 activity by direct interaction that subsequently results in SHP-1-mediated inhibition of p-STAT3.

**Dovitinib is effective in sorafenib-resistant cells**

Sorafenib is the first and only approved targeted therapy in HCC. However, many patients with HCC show resistance to sorafenib therapy. We established 2 sorafenib-resistant HCC cell lines (Huh-7-SR1 and Huh-7-SR2) by chronic exposure to sorafenib at low doses escalating to higher doses for a long period of time. As shown in Fig. 5A, Huh-7-SR1 and Huh-SR2 were resistant to sorafenib-induced apoptosis (10 μmol/L) and had much higher expression of p-STAT3 than wild-type cells. We next examined various STAT3 signaling pathway–related molecules in sensitive and resistant cells. In addition to p-STAT3, our data showed that both
resistant cell lines had higher expression of p-JAK1, p-JAK2, Mcl-1, and cyclin D1. Resistant cell lines had lower expression of SHP-1 and p-SHP-1 (Tyr 536). These data suggest that the STAT3 signaling pathway may be related to acquired resistance to sorafenib. As dovitinib inhibited p-STAT3 in HCC as described above, we next examined the effect of dovitinib in sorafenib-resistant cells. As shown in Fig. 5B, dovitinib downregulated p-STAT3 and induced apoptosis in Huh-7-SR1 and Huh-7-SR2 cells in a dose-dependent manner starting at a concentration of 5 μmol/L. Notably, dovitinib induced more significant downregulation of p-STAT3 and apoptosis than sorafenib in resistant cells, indicating that the effect of dovitinib on sorafenib-resistant cells may be due to inhibition of STAT3. Moreover, we examined the effect of dovitinib on the activity of SHP-1 in sorafenib-resistant cells. As shown in Fig. 4D, dovitinib significantly increased the activity of SHP-1, suggesting that dovitinib may reduce p-STAT3 through targeting SHP-1 in resistant cells. As sorafenib also affects SHP-1 in our previous studies (8, 9), it suggests that SHP-1 may not be the only modulator to mediate the effect of dovitinib in sorafenib-resistant cells. Other targets of dovitinib such as FGFR might also play a role in mediating the effects of dovitinib in overcoming sorafenib resistance. In addition, to investigate whether sorafenib plus dovitinib act synergistically, median effect analysis was conducted and showed that most combination index values were

Figure 4. Dovitinib increases phosphatase activity of SHP-1. A, left, SHP-1 activity in dovitinib-treated HCC cells. Cells were exposed to dovitinib (10 μmol/L) for 24 hours and then cell lysates were analyzed by phosphatase activity assay. Middle, effects of dovitinib on phosphatase activity in SHP-1-containing lysates. PLC5 cells were immunoprecipitated (IP) with anti-SHP-1 antibody. The lysates were incubated with dovitinib (10 nmol/L) for 30 minutes and then analyzed by SHP-1 phosphatase activity. Right, effects of dovitinib on phosphatase activity in recombinant SHP-1. Recombinant SHP-1 protein (25 ng) was incubated with dovitinib (10 nmol/L) for 30 minutes and then analyzed by SHP-1 phosphatase activity. Columns, mean; bars, SD (n = 6).

\[ P < 0.05. \]

B, effects of dovitinib on phospho-SHP-1 in HCC. Cells were exposed to dovitinib at the indicated concentrations for 24 hours. C, effect of dovitinib on SHP-1-overexpressed PLC5 cells. Cells were exposed to dovitinib (10 μmol/L) for 24 hours. Columns, mean; bars, SD (n = 6).

\[ P < 0.05. \]

D, SHP-1 activity in dovitinib-treated sorafenib-resistant HCC cells. Cells were exposed to dovitinib (10 μmol/L) for 24 hours and then cell lysates were analyzed by phosphatase activity assay. Columns, mean; bars, SD (n = 6).

\[ P < 0.05. \]
Dovitinib shows apoptotic effects in sorafenib-resistant HCC cells. A, establishment of sorafenib-resistant HCC cell lines (Huh-7-SR1 and Huh-7-SR2). B, left, expression levels of STAT3-related proteins in wide-type and sorafenib-resistant cell lines. Middle, Huh-7-SR1. Right, Huh-7-SR2. Dovitinib induced significant apoptosis and downregulated STAT3-related signals in resistant cells. C, left, PLC5 cells were treated with sorafenib and dovitinib for 24 hours administered over a range of concentrations at a fixed ratio of 1:2. After the percentage of apoptotic cells was determined in each condition, the combination index was calculated as described in Materials and Methods. CI value less than one is considered synergism. Middle, effects of dovitinib on apoptosis in primary acute leukemia cells. Cells were exposed to dovitinib at the indicated doses for 24 hours and apoptotic cells were determined by flow cytometry (sub-G1). Apoptotic cells were determined by flow cytometry. Columns, mean; bars, SD (n = 6). *P < 0.05; **P < 0.01. Right, effects of dovitinib on VEGFR2 in PLC5 cells. Cells were exposed to dovitinib at the indicated doses for 12 hours. D, effects of dovitinib on primary HCC cells. Cells were exposed to dovitinib at the indicated doses for 24 hours and apoptotic cells were determined by flow cytometry (sub-G1). Apoptotic cells were determined by flow cytometry. Columns, mean; bars, SD (n = 6). *P < 0.05; **P < 0.01. WT, wild-type.
less than one, indicating the combination was synergistic (Fig. 5C, left). Furthermore, we examined the effect of dovitinib in primary acute leukemia cells. Our data indicated that dovitinib induced apoptosis significantly and also downregulated p-STAT3 in these cells, suggesting that the effect of dovitinib on apoptosis and STAT3 is also present in primary cancer cells (Fig. 5C, middle). Next, dovitinib as a VEGFR inhibitor downregulated phospho-VEGFR2 (Tyr 1175) and p-STAT3 in PLC5 cells in a dose-dependent manner (Fig. 5C, right). Moreover, we examined the effect of dovitinib in primary HCC cells. As shown in Fig. 5D, dovitinib also downregulated p-STAT3 and induced significant apoptotic cell death in primary HCC cells, indicating that the effect of dovitinib on STAT3 may be clinically relevant.

**Effects of dovitinib in Huh-7 and PLC5 xenograft nude mice**

To assay whether the biologic effect of dovitinib in HCC cells is potentially clinically relevant, we tested the

![Figure 6](image_url)
dovitinib-treated Huh-7 and PLC5 tumors. Treatment with dovitinib significantly increased SHP-1 activity in both Huh-7 (Fig. 6A, right bottom) and PLC5 tumors (Fig. 6B, right bottom). Together, these data indicate that dovitinib inhibited tumor growth through SHP-1–dependent STAT3 inactivation.

Discussion

In this study, we showed that dovitinib affected SHP-1 activity directly and mediated the inactivation of STAT3 to induce HCC cell death. We thus suggest that the action of dovitinib is through an SHP-1–mediated downregulation of STAT3. Although there are still no patient-reported outcomes available for dovitinib treatment, our finding provides new mechanistic insight that may aid the ongoing dovitinib clinical trials. Dose-dependent treatment with dovitinib resulted in significant apoptosis, and downregulation of STAT3 that was essential for dovitinib-induced cell death (Figs. 1 and 2). Dissection of the molecular events associated with dovitinib treatment found that the apoptotic effect and downregulation of STAT3 could be rescued by silencing SHP-1. The possibility that dovitinib interacts with SHP-1 directly to increase phosphatase activity provides a novel mechanistic insight into the action of dovitinib in HCC (Fig. 4). In addition, surprisingly, we observed that dovitinib is more efficient in HCC than in other previously reported tumors. For example, a dose–response study of dovitinib-treated human colorectal cancer showed that growth inhibition was observed with daily oral administration of dovitinib at a dose of 60 to 100 mg/kg/d (22). Also, dovitinib was reported to impair mammary tumor growth at doses of 40 mg/kg/d (5). Our HCC-bearing xenograft group received only 15 mg/kg/d treatment with dovitinib but still showed strong tumor growth inhibition (Fig. 6). The reason why dovitinib showed better effect in our experiments may be due to early treatment. As we started the treatment of dovitinib in mice when the tumors were small, our in vivo data mainly indicated that dovitinib were able to slow down the tumor progression in HCC xenograft. To translate our work into clinical implications, we need to further examine the effect of dovitinib in tumors with larger sizes to find out whether dovitinib have effects on tumor regression. This finding implies that dovitinib has potential for use in targeted therapy for HCC. In addition, the STAT3-based molecular effects point to a more specific strategy for the application of RTK inhibitors.

The recent disclosure of the role of STAT3 signaling in carcinogenesis may contribute to the design of novel targeted therapies. Also, STAT3 is critical for the tumorigenic inflammatory microenvironment. In HCC, STAT3 frequently correlates with the constitutive upregulation of Ras implicated in HCC progression (23). In addition, several STAT3-driven proteins, such as survivin, are also upregulated in HCC and participate in cell proliferation (24). During the progression of glioblastoma, STAT3 is also thought to be involved in the maintenance of self-renewal and tumorigenic potential of glioblastoma stem cells (25). In our previous studies, we found that inhibition of STAT3 is crucial in molecular targeted therapies for HCC (8, 9). On the basis of kinase-independent derivatives modified from sorafenib, we previously concluded that STAT3 is a major target of sorafenib (9). All the above evidence suggests that STAT3-related inhibition enhances antitumor effect. In this study, downregulation of p-STAT3 was also found to be associated with the biologic effect of dovitinib on HCC. As yet, however, we cannot determine whether or not the alteration of STAT3 activity by dovitinib is dependent on kinase inhibition.

Regulation of the RTKs and PTPs, which occurs by reversible alteration in the phosphorylation state of specific tyrosine residues, results in various cellular events including alterations in signaling pathways and cell proliferation. Abnormal regulation of these kinases/PTPs contributes to defective signaling, which is associated with many pathologies including development of cancer. SHP-1, a PTP, is largely expressed in hematopoietic cells. So far, however, few reports have addressed the role of SHP-1 in tumor progression. Previous studies have suggested that this potential tumor suppressor is found in several cancer types (17). For example, SHP-1 protein expression was found to be dramatically downregulated in lymphoma and leukemia cell lines and was regarded as a characteristic of pathogenesis (26). The impaired function of SHP-1, which downregulates intracellular signaling transmembrane receptors including growth factors and cytokine receptors causing abnormal pathologies has been correlated with cancer (27). Most estrogen receptor (ER)-negative breast cancer cell lines were found to have very low or undetectable expression of SHP-1. Meanwhile, SHP-1 mRNA expression was normal in ER-positive breast cancer cells relative to breast epithelial cells (28). Taken together, these findings suggest that SHP-1 protein expression is largely repressed in most lymphocytic-related cancers, as well as in some nonlymphocytic tumor types.

According to our data, dovitinib may enhance the activity of SHP-1 through direct interactions (Fig. 4A). SHP-1 is composed of a catalytic domain at the C-terminal and 2 SH2 domains at the N-terminal for phosphotyrosine binding. Previously, the crystal structure of SHP-1 revealed an autoinhibitory confirmation between the SH2 domain at the N-terminal and the catalytic PTP domain (29, 30). The catalytic PTP loop for autoinhibition is critical for phosphatase activity according to these structure-based studies of SHP-1. It is possible that dovitinib enhances the activity of SHP-1 by interacting directly with the PTP loop to reduce the autoinhibition. More experiments need to be done to further explore the mechanism. Moreover, we observed that induction of SHP-1 activity mediates an important biologic effect in dovitinib-treated HCC cells. Upregulation of SHP-1 activity sensitizes cells to apoptosis in vitro and in vivo. The dovitinib-induced direct effect on SHP-1 implies that the boundaries of...
Inhibition may be altered by small molecules such as dovitinib. The multiple kinase inhibitor sorafenib is the first and only targeted therapy approved for HCC; however, the poor response rate renders the drug less than satisfactory. Clinically applicable regimens for overcoming sorafenib resistance are not yet available. STAT3 is a key factor in the mechanism of sorafenib resistance (Fig. 5). Moreover, STAT3-related kinases or downstream effectors, such as p-JAK1, p-JAK2, Mcl-1, and cyclin DI, are also upregulated in resistant cells. These results confirm that JAK/STAT3 signaling pathway is a vital modulator of the efficacy of sorafenib. Interestingly, decreased expression of SHP-1 was also found in sorafenib-resistant cells (data not shown). Collectively, these findings verify the role of SHP-1–correlated STAT3 signaling in HCC. We propose that targeting the SHP-1/STAT3 pathway may be an effective strategy for HCC treatment. In addition, the targeting of STAT3 by dovitinib in combination with sorafenib sensitized Huh-7–based sorafenib-resistant cells, suggesting that a dovitinib-guided strategy may be useful for overcoming sorafenib resistance.

In summary, our findings indicate that dovitinib-induced kinase inhibition results in a significant biologic effect via SHP-1–mediated blockade of p-STAT3. Also, in vivo, suppression of both Huh-7 and PLC5 xenograft tumors by dovitinib suggests the potential usefulness of dovitinib in the clinic. SHP-1–mediated STAT3 inhibition provides a potential target for future HCC molecular therapy.

Disclosure of Potential Conflicts of Interest

A.-L. Cheng serves as a consultant for Novartis. No potential conflicts of interests were disclosed by other authors.

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