Inhibition of Akt Reverses the Acquired Resistance to Sorafenib by Switching Protective Autophagy to Autophagic Cell Death in Hepatocellular Carcinoma

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Abstract
Sorafenib is the standard first-line systemic drug for advanced hepatocellular carcinoma (HCC), but the acquired resistance to sorafenib results in limited benefits. Activation of Akt is thought to be responsible for mediating the acquired resistance to sorafenib. The present study aims to examine the underlying mechanism and seek potential strategies to reverse this resistance. Two sorafenib-resistant HCC cell lines, which had been established from human HCC HepG2 and Huh7 cells, were refractory to sorafenib-induced growth inhibition and apoptosis in vitro and in vivo. Sustained exposure to sorafenib activated Akt via the feedback loop of mTOR but independent of protein phosphatase 2A in HCC cells. Autophagy participated in the resistance to sorafenib as an inhibition of autophagy reduced the sensitivity of sorafenib-resistant HCC cells to sorafenib, whereas activation of autophagy by rapamycin had the opposite effect. However, rapamycin did not show a synergistic effect with sorafenib to inhibit cell proliferation, while it also activated Akt via a feedback mechanism in sorafenib-resistant HCC cells. Inhibition of Akt reversed the acquired resistance to sorafenib by switching autophagy from a cytoprotective role to a death-promoting mechanism in the sorafenib-resistant HCC cells. Akt inhibition by GDC0068 synergized with sorafenib to suppress the growth of sorafenib-resistant HCC tumors that possessed the sorafenib-resistant feature in vivo. The results have provided evidence for clinical investigation of GDC0068, a novel ATP-competitive pan-Akt inhibitor, as the second-line treatment after the failure of sorafenib-mediated molecular targeted therapy for advanced HCC. Mol Cancer Ther; 13(6); 1589–98. ©2014 AACR.

Introduction
Hepatocellular carcinoma (HCC), the second most frequent cause of cancer death in men worldwide (1), is notoriously resistant to systemic chemotherapy (2). After searching for effective agents to combat HCC for decades, sorafenib has opened a window of hope and presents as the standard first-line systemic drug for advanced HCC (3). However, the promising treatment has demonstrated limited survival benefits with very low response rates (3, 4), and some patients with HCC initially respond to sorafenib but eventually the disease progresses (4), indicating that the resistance to sorafenib is common in HCC.

As a multitargeted kinase inhibitor, sorafenib targets the Raf/mitogen-activated protein kinase (MAPK)/extracellular signaling-regulated kinase (ERK) signaling pathway, and inhibits a number of tyrosine kinase receptors, including VEGF receptor, platelet-derived growth factor receptor, and c-Kit (3). The phosphoinositide 3-kinase (PI3K)/Akt pathway regulates a large number of molecules involved in all aspects of cancer progression (5), and is involved in the development and progression of HCC (6). Sorafenib activates Akt and upregulates its downstream factors such as ribosomal protein S6 kinase (S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) in HCC cells (7, 8). Sorafenib-resistant HCC cells have increased expression of phosphorylated Akt (p-Akt; ref. 9). There exist cross-talks between the PI3K/Akt and MAPK/ERK pathways (10), indicating that the latent compensatory mechanism of the PI3K/Akt pathway may contribute to sorafenib resistance in HCC. However, the mechanisms that underlie the role of Akt activation in this resistance remain unclear.

Induction of cell death is the final goal for developing anticaner agents. Autophagy was initially referred as a self-digestion process by which cytoplasmic contents are sequestered in autophagosomes and delivered to lysosomes for degradation (11), but now autophagy is also...
considered to be type II programmed cell death (PCD; ref. 12). Apoptosis, the type I PCD, is undoubtedly a tumor suppressing pathway, whereas autophagy is a double-edged sword depending on the cellular context and nature of the stimuli (13). The autophagic pathway cross-talks with apoptotic and other pathways, and the mutable molecular nodes in such pathways offer opportunities for therapeutic intervention (13). Autophagy-related protein (ATG) 6 (also known as Beclin 1) binds to other components to form a core complex to allow autophagosome nucleation (14), and interacts with apoptotic molecules (13). The PI3K/Akt pathway inhibits autophagy by activating mTOR (15), which exerts an inhibitory effect on autophagy by dysregulating Beclin 1 and ATG8 (microtubule-associated protein 1 light chain 3, LC3; ref. 16). These data indicate that activation of Akt may be responsible for mediating the acquired resistance to sorafenib by regulating the autophagic pathway.

Sorafenib promotes autophagic death of HCC cells (17), but contrary results have also been reported (18). Activation of autophagy is involved in the resistance to cisplatin (19), whereas inhibition of autophagy enhances the effect of sorafenib (20, 21) in HCC cells. However, the role of autophagy and its cross-talk with other pathways in mediating resistance to sorafenib of HCC have not yet been reported. Here, we demonstrate, for the first time, that autophagy switches from a cytoprotective function to a death-promoting role and drives the acquired resistance to sorafenib, while inhibiting Akt reverses the acquired resistance to sorafenib by activating the autophagic pathway in sorafenib-resistant HCC cells.

**Materials and Methods**

**Cell culture, antibodies, and reagents**

Human HCC HepG2 cells were obtained from the American Type Culture Collection, and Huh7 cells from Chinese Academy of Sciences Cell Bank (Shanghai, China) in 2009. Cells were immediately expanded, and multiple aliquots were cryopreserved and used within 3 months after resuscitation. No further authentication was done by the authors. Cells were cultured at 37°C in Dulbecco’s Modified Eagle Medium (Gibco BRL) supplemented with 10% FBS. The antibodies (Abs) against Akt, p-Akt (Ser473), ERK, p-ERK (Thr202/Thr204), glycogen synthase kinase (GSK)-3β, phosphorylated GSK3β (p-GSK3β; Ser9), mTOR, S6K, phosphorylated S6K (p-S6K; Thr389), 4EBP1, phosphorylated 4EBP1 (p-4EBP1; Ser65), PARP, LC3, Beclin1, ATG5, class III PI3K vascular protein sorting 34 (Vps34), UV radiation resistance-associated gene (UVRAG), and p62 were purchased from Cell Signaling Technology. The Abs against BAD, caspase-3 and -9, p27, cyclin D1, Bcl-2, and β-actin were from Santa Cruz Biotechnology. The anti–Ki-67 Ab was from Abcam. Sorafenib and perifosine were from Jinan Trio Pharmatech Biotechnology. The Abs against Bad, caspase-3 and -9, sorting 34 (Vps34), UV radiation resistance-associated (PARP), LC3, Beclin1, ATG5, class III PI3K vascular protein sorting 34 (Vps34), UV radiation resistance-associated (UVRAG), and p62 were purchased from Cell Signaling Technology. The Abs against BAD, caspase-3 and -9, p27, cyclin D1, Bcl-2, and β-actin were from Santa Cruz Biotechnology. The anti–Ki-67 Ab was from Abcam. Sorafenib and perifosine were from Jinan Trio Pharmatech Co., Ltd. Rapamycin (RAP), 3-methyladenine (3-MA), bafilomycin A1 (Baf-A1), okadaic acid (OA), and 1,9-dideoxy forskolin (Forskolin) were from Sigma-Aldrich. Sorafenib, GDC0068, rapamycin, and Baf-A1 were dissolved in dimethyl sulfoxide to make a stock solution of 100 mmol/L, 100 mmol/L, 1 mmol/L, and 1 mmol/L for in vitro assays, respectively. Perifosine was dissolved in PBS to make a stock solution of 30 μmol/L. 3-MA was dissolved in PBS at a concentration of 200 mmol/L by heating to 60 to 70°C immediately before use. For animal experiments, sorafenib was suspended in the vehicle solution containing Cremophor (Sigma-Aldrich), 95% ethanol and water in a ratio of 1:1:6 (22), and GDC0068 was dissolved in 0.5% methylcellullose/0.2% Tween-80 (23). The PI (propidium iodide)/Annexin V-FITC apoptosis detection kit was from BD Biosciences. The Cell Counting Kit-8 (CCK-8) kit was from Dojindo Laboratories. Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling agent (TUNEL) was from Roche.

**Establishment of sorafenib-resistant cells**

The IC50 of HCC cells to sorafenib was initially determined by incubating cells with different concentrations of sorafenib in 96-well plates, and cell viability was measured 3 days later as described below. The cells were cultured in 6-well plates at 1 × 104 cells/well and incubated with sorafenib at a concentration just below their respective IC50. The concentration of sorafenib was slowly increased by 0.25 μmol/L per week. After 6 to 7 months, two sorafenib-resistant cell lines were obtained, termed HepG2-SR and Huh7-SR, respectively, and were continuously maintained by culturing them in the presence of sorafenib.

**Analysis of autophagy by GFP-LC3 redistribution**

The GFP tandem fluorescent-tagged LC3 expression plasmid was transfected into cells using Lipofectamine 2000 (24). LC3 redistribution was detected under a fluorescent microscope.

**PP2A phosphatase activity assay**

The activity of PP2A phosphatase in cell lysates was measured by using a PP2A immunoprecipitation phosphatase assay kit (Millipore).

**Animal experiments**

Six- to 8-week male nude BALB/c-nu/nu mice were obtained from the Animal Research Center, The First Affiliated Hospital of Harbin Medical University (Harbin, China). The study protocol had been approved by the Animal Ethics Committee of Harbin Medical University. The animal experiment schedule was designed on the basis of the preliminary experiments (Fig. 1). Briefly, Huh7-SR cells (5 × 106) were subcutaneously inoculated into the back of mice, which received daily oral administration of 15 mg/kg sorafenib. The use of lower dose of sorafenib was to maintain the sorafenib-resistant ability of Huh7-SR cells, which were kept in the presence of sorafenib in culture. Fifteen days later, another group of mice
received subcutaneous injections of Huh7 cells (5 × 10^6). Thirty days later, three Huh7 and three Huh7-SR tumors were harvested. The remaining mice were assigned to different treatments. Sorafenib was orally administered at a dose of 30 mg/kg daily, and GDC0068 orally at a dose of 25 mg/kg twice per week. The tumor volumes were measured every 3 days. The tumors were harvested at the end of experiments.

Cell viability analysis, in vitro apoptosis assay, visualization of apoptotic cells by laser scanning confocal microscopy, assessment of Ki-67 proliferation index, in situ detection of apoptotic cell, and immunoblotting

All these methods have been described in details previously (24–26).

Transfection of Akt siRNA

A double-strand siRNA targeting human Akt (5’-GUGUCAUGUACGAGAUGATT-3’ and 5’-UCAUCUCGUACAUGACCATTT-3’) encoding nucleotides 1006–1025 of Akt1 (GenBank: NM_001014431.1), nucleotides 1210–1229 of Akt2 (GenBank: XM_005336494.1), and nucleotides 955–974 of Akt3 (GenBank: XM_004691046.1) with two introduced thymidine residues at the 3’ end was produced by GenePharma Co., Ltd. The nonspecific scrambled siRNA (5’-UUCUCCGAACGUGACACGU-3’ and 5’-ACGUGACACGUUCGGAGAA-3’) served as a control. Cells were grown to 60% to 70% confluence, and incubated with siRNAs at a final concentration of 0.1 μmol/L by using Lipofectamine 2000 (Invitrogen) in a serum-free medium for 24 hours and then subjected to the assays.

Electronic microscopy

Cells or tissues were fixed in 2.5% glutaraldehyde solution for 1 hour, washed twice with PBS, followed by further fixation with 1% Osmic acid for 1 hour, dehydrated with a graded series of ethanol, embedded, and sectioned. Sections were stained with Uranium Acetate and Lead Citrate, and observed under a transmission electronic microscope (JEN-M1220, Toshiba; ref. 24).

Statistical analysis

The data were expressed as mean values ± SD. Comparisons were made using one-way ANOVA followed by Dunnet t test. P < 0.05 was considered significant.

Results

Sorafenib-resistant HCC cells are refractory to sorafenib-induced growth inhibition and apoptosis

After incubation with 10 μmol/L of sorafenib for 48 hours, the viability of HepG2-SR and Huh7-SR cells were 78.8% and 77.2%, respectively, which were significantly higher than that of HepG2 and Huh7 cells (18.4% and 31.9%, respectively; Fig. 2A). Even when the concentration of sorafenib reached 20 μmol/L, the viability of HepG2-SR and Huh7-SR still remained at 38.5% and 46.2%, respectively, whereas HepG2 and Huh7 cells were almost completely dead (Fig. 2A). The in vitro findings were supported by the in vivo data (Supplementary Fig. S1). Apoptotic rates of HepG2 cells were 4.1- and 3.9-fold higher than that of HepG2-SR cells, after exposure to 5 and 10 μmol/L of sorafenib, respectively (Fig. 2B and C). Apoptotic rates of Huh7 cells were 2.8- and 4.0-fold higher than that of Huh7-SR cells, after exposure to 5 and
of sorafenib, respectively (Fig. 2B and C). The apoptotic results were supported by the expression of two key apoptotic proteins, caspase-3 and PARP (Fig. 2D and E), indicating that sorafenib-resistant cells are refractory to sorafenib-induced apoptosis through caspase-dependent and -independent ways.

**Autophagy participates in the mechanisms for the resistance to sorafenib**

After incubation for 48 hours in the presence and absence of sorafenib (10 μmol/L), sorafenib-resistant cells expressed higher levels of p-Akt and different levels of its downstream factors, including p-GSK3β, mTOR, p-S6K and p-4EBP1, Bad, p27, and cyclin D1, compared with their respective parental cells (Supplementary Fig. S2A and Supplementary Table S1). The results indicate that sustained exposure to sorafenib activates the Akt pathway. In addition, sorafenib-induced phosphorylation of Akt was independent of protein phosphatase inactivation (Supplementary Fig. S2B and S2C). Given that mTOR is a gatekeeper of autophagy by dysregulating ATGs (16), we hypothesized that autophagy may participate in the acquired resistance to sorafenib. Accordingly, sorafenib-resistant cells expressed lower levels of LC3-II, ATG5, Vps34 and Beclin 1, and expressed higher levels of P62 and Bcl-2, than their respective parental cells in either the presence or absence of sorafenib (Fig. 3A). Sorafenib upregulated
the expression of LC3-II, ATG5, Beclin 1, and Vps34, downregulated the expression of P62, and had little effects on UVRAG and Bcl-2, in either parental or sorafenib-resistant cells (Fig. 3A). The main in vitro findings were further supported by the in vivo results (Supplementary Fig. S3).

We next showed that inhibition of autophagy by 3-MA and Baf-A1 protected HepG2-SR and Huh7-SR cells against sorafenib-induced reduction in cell viability (Fig. 3B and C). In contrast, rapamycin, an inhibitor of mTOR and an inducer of autophagy (27), enhanced sorafenib-induced growth inhibition in a dose-dependent manner (Fig. 3D). However, the synergistic effects of rapamycin and sorafenib were not significant, as the values for the coefficient of drug interaction (CDI; refs. 25, 28) for HepG2-SR and Huh7-SR cells treated with 1, 10, or 100 nmol/L of rapamycin in the presence of sorafenib (10 μmol/L) were all more than 0.7.

Figure 3. Autophagy affects the sensitivity of sorafenib-resistant HCC cells to sorafenib. A, lysates of HepG2, HepG2-SR, Huh7, and Huh7-SR cells incubated with 0, 5 or 10 μmol/L of sorafenib for 48 hours were immunoblotted to detect expression of autophagy-associated proteins. B–D, the above cells were incubated for 48 hours in the presence or absence of sorafenib (10 μmol/L in B and D or 15 or 20 μmol/L in C), and/or 3-MA (3-methyladenine; 10 mmol/L), and/or Baf-A1 (bafilomycin A1, 50 nmol/L), or with different concentrations of rapamycin (D). Cell viability (%) was compared with the corresponding untreated cells. E, immunoblot analyses of lysates of Huh7-SR cells from B. F, immunoblot analyses of lysates of Huh7-SR cells from D. The band density of LC3-II was normalized to LC3-I, and that of other proteins was normalized to β-actin. Data represent three independent experiments. The statistical comparison was performed for sorafenib-resistant cells only. *, P < 0.05 and **, P < 0.001 versus respective untreated cells; †, P < 0.05 and ††, P < 0.001 versus sorafenib alone; ‡, P < 0.05 and ‡‡, P < 0.001 versus rapamycin alone. øø, P < 0.001.
3-MA downregulated the expression of LC3-II and Beclin 1, whereas Baf-A1 upregulated LC3-II and downregulated Beclin 1, in Huh7-SR cells (Fig. 3E). Both 3-MA and Baf-A1 increased the expression of pro-caspase-3 but had little effect on cleaved-PARP expression (Fig. 3E). Rapamycin upregulated the expression of LC3-II, Beclin 1, p-Akt, and cleaved-PARP, and increased the activation of caspase-3 (Fig. 3F), indicating that induction of autophagy by rapamycin promotes apoptosis, but also results in activation of Akt, in sorafenib-resistant cells.

Inhibition of Akt synergizes with sorafenib to inhibit cell viability, promote apoptosis, and induce autophagic cell death in sorafenib-resistant cells

Inhibition of Akt by GDC0068 synergized with sorafenib to reduce the viability of HepG2-SR and Huh7-SR cells in dose- and time-dependent manners (Fig. 4A and Supplementary Fig. S4A). The CDIs for HepG2-SR cells treated with 1, 5, or 10 μmol/L of GDC0068 in the presence of sorafenib (10 μmol/L) were 0.67, 0.63, or 0.65, respectively; and that for Huh7-SR were 0.79, 0.59, or 0.61, respectively, indicating that the synergistic effects were significant. The effects of Akt inhibition on cell viability were further supported by application of perifosine, another Akt inhibitor (Supplementary Fig. S4B). GDC0068 also showed a significantly synergistic effect with sorafenib in inducing cell apoptosis with CDIs of 0.48 and 0.53, in HepG2-SR and Huh7-SR cells, respectively (Fig. 4B and C).

Sorafenib and GDC0068 upregulated, and their combination showed an even stronger effect in increasing the expression of LC3-II and Beclin 1 in Huh7-SR cells (Fig. 4D). Inhibition of Akt by perifosine or depletion of Akt by siRNAs had a similar effect on the expression of autophagic proteins (Supplementary Fig. S4C and S4D). Consequently, the number of GFP-LC3–positive dots in cells treated with sorafenib or GDC0068 were significantly higher than untreated cells, and their combination resulted in the highest number of GFP-LC3–positive dots (Fig. 4E). The CDI for GDC0068 and sorafenib to induce...
autophagy of Huh7-SR cells was 0.51, indicating that the synergistic effect was significant. The treated cells showed that GFP-LC3 signals shifted from a diffuse cytoplasmic pattern to a punctate membrane pattern, suggesting the formation of autophagic vacuoles (Fig. 4F). Electron microscopy revealed abundant autophagic vacuoles in sorafenib or GDC0068-treated cells, but scarcely in untreated cells; and the combinational therapy resulted in the most autophagosomes (Fig. 4G).

**GDC0068 synergizes with sorafenib to suppress sorafenib-resistant tumors**

Administration of sorafenib and GDC0068 into the mice reduced the size of Huh7-SR tumors by 15.8% and 37.5%, respectively, and the combination therapy further reduced the size by 71.3%, compared with vehicle-treated tumors at day 15 (Fig. 5A).

Immunoblotting analysis of tumor lysates demonstrated the similar alteration of expression of p-Akt, LC3-II, pro–caspase-3, and PARP (Fig. 5B), as shown in vitro (Supplementary Fig. S5A–S5C). Electron microscopy also revealed abundant autophagosomes in cells from tumors treated by sorafenib plus GDC0068, but scarcely in vehicle-treated tumors (Fig. 5C). There were fewer Ki-67–positive cells in tumors treated with sorafenib or GDC0068, compared with vehicle-treated tumors; and the combination therapy resulted in even fewer Ki-67–positive cells (Fig. 5D and E). Tumors treated with sorafenib and GDC0068 had a greater number of TUNEL-positive cell than vehicle-treated tumors, and the combination therapy resulted in even more TUNEL-positive cells (Fig. 5D and F).

**Discussion**

Considering the dilemma that no effective systemic therapy for HCC is available so far after failure of sorafenib therapy (29), sorafenib remains in a unique position for the treatment of HCC. The present study has demonstrated that activation of Akt induced by chronic exposure of sorafenib regulates the acquired resistance to sorafenib by switching autophagy from a cytoprotective role to a death-promoting mechanism in HCC cells.

Although it is not a direct target of sorafenib, the PI3K/Akt pathway plays an important role in sorafenib

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**Figure 5. Inhibition of Akt overcomes sorafenib resistance in vivo.** A, subcutaneous tumors were established in mice that received different treatments for 15 days as described in Materials and Methods and Fig. 1. The size (mm³) of tumors was recorded. B, immunoblot analyses of lysates of tumors harvested from A at the end of experiments. C, sections of tumors treated with vehicle or sorafenib + GDC0068 were viewed under electron microscopy. Arrows point to autophagosomes (magnification, ×10,000); D, representative images from tumor sections stained with an anti–Ki-67 Ab (top) or TUNEL (bottom); magnification, ×200). Proliferation index (E) and apoptosis index (F) were quantified, n, the number of samples examined; *, P < 0.05 and **, P < 0.001 versus vehicle-treated tumors; †, P < 0.001 versus sorafenib alone; ###, P < 0.001 versus GDC0068 alone.
resistance because it crosstalks with the MAPK/ERK pathway (10). Many lines of studies have shown that sorafenib activates the PI3K/Akt pathway (7, 8, 18, 30), and blockage of this pathway enhances the efficacy of sorafenib (7, 18, 30), but it has also been reported that sorafenib enhances proteasome inhibitor-induced cell death by inactivating Akt in HCC (31), indicating that the complicated role of the PI3K/Akt pathway in sorafenib resistance is far from clear. Here, we have demonstrated that Akt was activated in sorafenib-resistant cells, in accordance with a previous report (9). Although phosphorylation of Akt can be induced by protein phosphatase inactivation (32), the present study showed that the sorafenib-induced Akt phosphorylation was independent of protein phosphatase 2A (PP2A), a major serine/threonine phosphatase in eukaryotic cells (33). In accord, the synergistic interaction between sorafenib and bortezomib, but not sorafenib alone, involved PP2A-dependent Akt inactivation in HCC cells (34). Akt regulates the expression and phosphorylation of mTOR, GSk3β, Bad and p27; mTOR regulates the apoptotic proteins S6K and 4EBP1, and the autophagic proteins LC3 and Beclin 1; and GSk3β regulates cyclin D1 and caspase-9 (refs. 5, 13, 29, 35; Fig. 6). Therefore, specific inhibition of Akt could downregulate the above downstream factors, thus inducing apoptosis and inhibiting proliferation of HCC cells.

Among the over hundred molecules in the PI3K/Akt pathway, mTOR interacts with the MARK, apoptotic, and autophagic pathways and has been most extensively studied, and several mTOR inhibitors are under evaluation in clinical trials (36). Inhibition of mTOR enhances the chemosensitivity (37), yields antiproliferative effects in both parental and sorafenib-resistant HCC cells (35), and augments the effects of sorafenib in a syngeneic orthotopic model of HCC (32). However, here we found that rapamycin did not show a synergistic effect with sorafenib in inhibiting the growth of sorafenib-resistant HCC cells despite the higher basal expression of mTOR (8). The unhampered feedback loop driving PI3K/Akt pathway by mTOR inhibition has been recently reported, and inhibition of Akt interrupted this rapamycin-induced feedback loop, thereby enhancing the antiproliferative effects of mTOR inhibition (38). Accordingly, here we have shown that chronic exposure of sorafenib inhibited activation of ERK (9), sequentially inhibited mTOR independent of the PI3K/Akt pathway (39), resulting in activation of Akt via the feedback loop in sorafenib-resistant HCC cells (Fig. 6). This may explain that the effect of mTOR inhibition by rapamycin is not ideal to reverse sorafenib resistance.

The role of autophagy as a target in cancer therapy remains controversial (40). It has been reported that sorafenib induced autophagic death of HCC cells (17).
However, the authors used a high concentration of sorafenib at 20 μM/L (18). Even with the same concentration of sorafenib, another study showed that autophagy protected against sorafenib-induced cell death (20). Here, we have demonstrated that sorafenib at 10 μM/L activated autophagy, which played a protective role against cell death of parental HCC cells, and is in agreement with a previous report (21); but exhibited a death-promoting role in sorafenib-resistant HCC cells. Autophagy induced by vorinostat, a histone deacetylase inhibitor that has been approved for treating cutaneous T-cell lymphoma in clinic (41), protected against cell death independent of mTOR in vorinostat-resistant lymphoma cells (42). The autophagic pathway crosstalks with both the caspase-dependent and -independent apoptotic pathways (13, 21, 43). Sustained drug exposure could induce imbalanced apoptotic pathways, leading to the resistance to apoptosis (44). When the apoptotic pathway is suppressed, autophagy as an adaptive response will switch from a protective role to a death-promoting function through the existing cross-talk with the apoptotic pathway (13, 45). This explanation is supported by a recent study that paclitaxel resistance is associated with switch from apoptotic to autophagic cell death in breast cancer cells (46).

The mechanisms accounting for the acquired resistance to sorafenib in HCC remain complex (47). The detailed cellular signaling pathways and their interactions in parental and sorafenib-resistant HCC cells, and the interventions with agents tested in the present study are summarized in Fig. 6. Briefly, autophagy plays a protective role against sorafenib-induced cell death in the parental cells. In sorafenib-resistant cells, sustained exposure to sorafenib activates Akt directly or via the feedback loop of mTOR, which is inhibited by sorafenib through the ERK pathway (Fig. 6). Sorafenib induces autophagy independent of the ERK pathway (20). Activation of autophagy by rapamycin partially reverses the acquired resistance to sorafenib as rapamycin-mediated mTOR inhibition in turn activates Akt via the feedback loop. However, inhibition of Akt by GDC0068 significantly enhances the efficacy of sorafenib to suppress the growth of sorafenib-resistant HCC cells by inducing autophagic cell death. As a novel ATP-competitive pan-Akt inhibitor, GDC0068 binds to the active site of Akt and protects it from phosphatases, leading to increased Akt phosphorylation (48, 49). It has been reported that GDC0068 has less dose-limiting toxicity and is more effective in inhibiting the function of Akt than allorsteric Akt inhibitors (48, 49). GDC0068 has displayed anticancer activities against several types of cancers (49). The results presented warrant clinical investigation of GDC0068 as the second-line treatment after the failure of sorafenib to treat advanced HCC.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: H. Qiao, H. Jiang, X. Sun
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