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A novel obatoclax derivative, SC-2001, induces apoptosis in hepatocellular carcinoma cells through SHP-1-dependent STAT3 inactivation

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ABSTRACT

We investigated the effects of a novel compound, SC-2001, on hepatocellular carcinoma (HCC). SC-2001, which is structurally related to the Mcl-1 inhibitor obatoclax, showed better antitumor effects than obatoclax in HCC cell lines, including HepG2, PLC5 and Huh-7. Like obatoclax, SC-2001 inhibited the protein–protein interactions between Mcl-1 and Bak. However, SC-2001 downregulated the protein levels of Mcl-1 by reducing its transcription whereas obatoclax had no significant effect on Mcl-1 expression. As Mcl-1 is regulated by signal transducers and activators of transcription 3 (STAT3), we found that SC-2001 downregulated the phosphorylation of STAT3 (Tyr 705) and subsequently inhibited transcriptional activities of STAT3 in a dose-dependent manner. In addition to Mcl-1, STAT3-regulated proteins, including survivin and cyclin D1, were also repressed by SC-2001. Notably, SC-2001 reduced IL-6-induced STAT3 activation in HepG2 and PLC5 cells. Ectopic expression of STAT3 abolished the prominent apoptotic death in SC-2001-treated PLC5 cells, indicating that STAT3 is indispensable in mediating the effects of SC-2001. Importantly, SC-2001 enhanced the expression of SHP1, a negative regulator of STAT3. Inhibition of SHP-1 by either specific inhibitor or small interference RNA reduced the apoptotic effects of SC-2001, indicating that SHP-1 plays a key role in mediating SC2001-induced cell death. SC-2001 enhanced the activity of SHP-1 in all tested HCC cells including HepG2, PLC5 and Huh-7. Finally, SC-2001 reduced PLC5 tumor growth, downregulated p-STAT3 and upregulated SHP-1 expression and activity *in vivo*. In conclusion, our results suggest that SC-2001 induces apoptosis in HCC, and that this effect is mediated through SHP-1-dependent STAT3 inactivation.

1. Introduction

Advanced or recurrent hepatocellular carcinoma (HCC) is frequently resistant to conventional chemotherapeutic agents and radiation and thus remains one of the most difficult cancers to treat [1,2]. The discovery of targeted agents with tolerable toxicity is, therefore, mandatory to advance anti-HCC therapy [2]. The success of sorafenib, a multi-targeted receptor tyrosine kinase (RTK) inhibitor, in two randomized controlled phase III trials for advanced HCC [3,4] supports the use of molecularly targeted therapies in the treatment of advanced HCC.

One of the chief reasons that HCC cells are resistant to chemotherapy is their ability to resist apoptosis [5]. One important mechanism of apoptosis-resistance involves the dominant apoptosis regulatory protein family, B-cell lymphoma 2 (Bcl-2). Bcl-2

Abbreviations: HCC, hepatocellular carcinoma; STAT3, signal transducers and activators of transcription 3; SOCS, suppressor of cytokine signaling; SHP-1, Src homology region 2 domain-containing phosphatase 1; PARP, poly ADP-ribose polymerase; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; NMR, nuclear magnetic resonance; HRMS, high-resolution mass spectrometry; LC/MS, liquid chromatography/mass spectrometry; ESI, electrospray ionization.

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family members are key regulators of the intrinsic apoptotic pathway and include both antiapoptotic (Bcl-2, Mcl-1 and Mcl-xL, *etc.*) and proapoptotic (such as BH3-only BIM and the apoptotic effectors BAX and BAK, *etc.*) proteins [6]. The antiapoptotic proteins of the BCL-2 family are overexpressed and dysregulated in various cancers, including HCC [5]. Bcl-xL and Mcl-1 in particular play significant cytoprotective roles in HCC [7–10], therefore, Bcl-2 proteins have emerged as attractive targets for novel anticancer drugs [6]. A common strategy in the design of Bcl-2 protein inhibitors is based on mimicking the actions of endogenous inhibitors that bind antiapoptotic Bcl-2 proteins via the Bcl-2 homology 3 (BH3) domains (BH3 mimetics) [11]. Obatoclax (GX15–070, Gemin X Pharmaceuticals) is a small molecule pan-Bcl-2 inhibitor that acts as a BH3-mimetic to disrupt the interactions of anti-apoptotic and proapoptotic proteins, such as Mcl-1 and Bak, and has shown early evidence of efficacy and safety in several phase I trials [12–15]. Obatoclax is currently being tested both alone and in combination with other chemotherapeutics in phase II trials in hematological malignancies and solid tumors [6]. Therefore, obatoclax is considered a promising agent for hepatobilliary cancers [5].

It is notable that some important antiapoptotic members of the Bcl-2 members family, such as Bcl-xL and Mcl-1, can be regulated by oncogenic transcription factors, such as STAT3. STAT3 is crucial in the regulation of genes involved in cell proliferation and survival, and is constitutively activated in common human cancers, including HCC [16]. Upon stimulation by cytokines, growth factors or hormones, STAT3 is phosphorylated (activated) and homodimerizes or heterodimerizes with STAT1 in the cytoplasm and then translocates to the nucleus. In cancer cells, constitutively activated STAT3 directly contributes to tumorigenesis, invasion, and metastasis [16]. Downregulation of STAT3 activity has been shown to be a promising anti-HCC strategy [16–18]. Recently, we also found that sorafenib inhibited p-STAT3, which contributed to its anti-HCC efficacy [19,20]. Interestingly, a number of protein tyrosine phosphatases have been shown to negatively regulate STAT3 signaling through direct dephosphorylation of p-STAT3 (Tyr 705); these include members of the SH2-domain containing tyrosine phosphatase family (SHP-1 and SHP-2), and protein tyrosine phosphatase 1B (PTP-1B). Loss of SHP-1 has been shown to enhance JAK3/STAT3 signaling in ALK-positive anaplastic large-cell lymphoma and in cutaneous T-cell lymphoma [21,22]. In addition, several agents such as betulinic acid [23], boswellic acid [24], and 5-hydroxy-2-methyl-1,4-naphthoquinone (a vit-K3 analogue) [25] that can enhance the SHP-1 pathway have shown anti-cancer potential. Therefore, activity of protein tyrosine phosphatases may be critical for the regulation of STAT3 phosphorylation in cancer cells.

In this study, we found that SC-2001, a novel Mcl-1 inhibiting compound which is structurally related to obatoclax, has more potent antitumor activity than obatoclax and has a novel drug mechanism (SHP-1-dependent STAT3 inactivation) that is distinct from obatoclax in HCC cells. We showed that SC-2001 not only inhibits the protein–protein interactions between Mcl-1 and Bak, but also downregulates the protein levels of Mcl-1 by reducing its transcription. We further discovered that SC-2001 downregulated the phosphorylation of STAT3 (Tyr 705) and subsequently inhibited the transcriptional activities of STAT3. Furthermore, we noticed that SC-2001 enhanced the expression of SHP-1, which played a key role in p-STAT3 downregulation and in mediating SC2001-induced apoptosis. Importantly, this SHP-1-dependent STAT3 inhibitory mechanism that mediates the efficacy of SC-2001 was confirmed in an *in vivo* nude mouse model. Our results suggest that SC-2001 is a novel STAT3 inhibitor that acts through enhancing SHP-1 activity and has promising anti-HCC efficacy.

2. Materials and methods

2.1. Synthesis, purification, and characterization of SC-2001

$PdCl_2$ (0.1 equiv, 59%) and PPh_3 (0.45 equiv) were added to a solution of toluene (1 mL). The mixture was stirred at 70 °C under nitrogen for 20 min and transferred into a flask with (Z)-2-((1H-pyrrol-2-yl)methylene)-3-methoxy-2H-pyrrol-5-yl trifluoromethanesulfonate (1.0 equiv), 5-bromo-1H-indol-2-ylboronic acid (1.2 equiv), solid sodium carbonate (1.0 equiv) and 10% water/dioxane (5 mL). The reaction mixture was stirred at 100 °C for 90 min, then poured into 10 mL water and extracted with ethyl acetate (20 mL) three times. The organic layer was collected, washed with brine, dried over $MgSO_4$ and concentrated. The crude product was collected by silica gel with the eluent ethyl acetate:hexane (1:20–1:5). SC-2001 compound was determined by nuclear magnetic resonance (NMR) and mass spectrometry (MS). ^1H NMR (400 MHz, $CDCl_3$) 7.65 (d, J = 1.6 Hz, 1H), 7.15 (dd, J = 8.8 Hz, J = 2.0 Hz, 1H), 7.07 (s, 1H), 6.88 (s, 1H), 6.83 (d, J = 8.4 Hz, 1H), 6.73 (s, 1H), 6.60 (d, J = 3.6 Hz, 1H), 6.22 (s, 1H), 6.12 (t, J = 3.2 Hz, 1H), 4.04 (s, 3H); LCMS (ESI): m/z 368.2 (100, M^+H^+); high-resolution mass spectrometry (HRMS) calculated for $C_{18}H_{14}BrN_3O$ (M^+H^+): 368.0393. Found 368.0345. Yield: 93%.

2.2. Reagents and antibodies

SHP-1 inhibitor was purchased from Cayman Chemical (Ann Arbor, MI). Antibodies for immunoblotting such as cyclin D1, and PARP were purchased from Santa Cruz Biotechnology (San Diego, CA). Other antibodies such as survivin, phospho-STAT3 (Tyr705), STAT3, SHP-1, SHP-2, survivin, and PTP-1B were from Cell Signaling (Danvers, MA).

2.3. 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) and HepG2 cell lines were obtained from American Type Culture Collection (ATCC, Manassas, VA). All cells obtained from HSRRB or ATCC were immediately expanded and frozen 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 conducted in our laboratory. Cells were maintained in DMEM supplemented with 10% fetal bovine serum (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% CO_2 in air.

2.4. Apoptosis analysis

The following methods were used to assess drug-induced apoptotic cell death: cell death detection ELISA for cytoplasmic histone associated DNA fragments (Roche, Indianapolis, IN), and western blot for PARP cleavage. Further, HCC cells were treated with DMSO or obatoclax or SC-2001 at the indicated dose for 12 h, and the specific enrichment of oligonucleosomes released into cytoplasm was quantified by cell death ELISA.

2.5. Reverse transcriptase PCR

Total RNA was isolated from cell lines with TRIzol (Invitrogen) and cDNA was prepared from 2 mg of RNA using a First-Strand Cdna Synthesis Kit according to the manufacturer's instructions (Amersham Biosciences, Amersham, UK). Oligonucleotide sequences were as follows: SHP-1, 5′-GCC CAG TTC ATT GAA ACC AC- 3′ (sense) and 5′-GAG GGA ACC CTT GCT CTT CT-3′ (antisense); GAPDH, 5′-CGA CCA CTT TGT CAA GCT CA-3′(sense) and 5′-AGG GGT CTA CAT GGC AAC TG-3′ (antisense); Mcl-1: 5′-CTT GCC ACT TGC TTT TCT GG-3′ (sense) and 5′-CAA GGC ATG CTT CGG AAA CT-3′ (antisense). The following PCR conditions were used: denaturation at 95 °C for 10 min followed by 35 cycles of 94 °C for 1 min, annealing for 1 min at 57 °C, and elongation for 1 min at 72 °C, and a final elongation step at 72 °C for 10 min.

2.6. Gene knockdown using siRNA

Smart-pool small interfering RNAs (siRNAs), including the control (D-001810-10) and SHP-1 were purchased from Dharmacon (Chicago, IL). The knockdown procedure was as described previously [26]. Briefly, PLC5 cells were transfected with siRNAs against the phosphatases given above or the control sequence for 48 h and then treated with SC-2001 at the indicated concentrations. The cell extracts were analyzed by western blot.

2.7. PLC5 cells with ectopic expression of STAT3

STAT3 cDNA (KIAA1524) was purchased from Addgene plasmid repository (http://www.addgene.org/). STAT3-overexpresed PLC5 cells derived from a single stable clone were prepared for evaluating the major target of SC-2001. Briefly, following transfection, cells were cultured in the presence of G418 (0.8 mg/mL)

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according to previous reports [27]. After 8 weeks of selection, surviving colonies, i.e., those arising from stably transfected cells were selected and individually amplified.

2.8. SHP-1 phosphatase activity

A RediPlate 96 EnzChek® Tyrosine Phosphatase Assay Kit (R-22067) was used for SHP-1 activity assay (Molecular Probes, Carlsbad, CA). The method was as described previously [20].

2.9. 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 performed in accordance with protocols approved by National Taiwan University. When PLC5 tumors reached 100 mm^3, mice received SC-2001 (10 mg/kg) orally. Controls received vehicle.

2.10. Statistical analysis

Data are expressed as mean ± SD or SE. Statistical comparisons were based on nonparametric tests and statistical significance was defined at $P < 0.05$. All statistical analyses were performed using SPSS for Windows version 12.0 software (SPSS, Chicago, IL).

3. Results

3.1. SC-2001 shows growth-inhibitory effect in HCC cells

SC-2001 is an analogue of obatoclax. To investigate the anticancer effects of SC-2001 (Fig. 1A), we first assessed growth inhibition in response to SC-2001 or obatoclax treatment in a panel of three human HCC cell lines: HepG2, PLC5, and Huh-7. Cell viability was determined by MTT assay after treatment for 48 h (up to 20 μM). As shown in Fig. 1B, SC-2001 significantly reduced cell viability in a dose-dependent manner in all three cell lines. Next, we examined the apoptotic effect of SC-2001 on HCC. Apoptotic effects were determined by DNA fragmentation ELISA assay. All HCC cell lines were exposed to SC-2001 or obatoclax at the same concentration. The data shown in Fig. 1C indicate that SC-2001 substantially increased apoptotic cell death and had a more potent growth inhibitory effect than obatoclax. We further confirmed the apoptotic effect of SC-2001 by detecting PARP cleavage. SC-2001 caused the cleavage of PARP in all tested cells (Fig. 1D). These data indicate that SC-2001 has more potent growth inhibition and a stronger apoptosis inducing effect than obatoclax in HCC cells.

3.2. SC-2001 downregulated Mcl-1 transcription

To elucidate the mechanism by which SC-2001 inhibits cell growth and induces apoptosis in HCC, we first examined the effect of SC-2001 on inhibition of Mcl-1-Bak interactions. Like obatoclax, SC-2001 reduced the interactions between Mcl-1 and Bak (Fig. 2A). We next examined the effects of SC-2001 on several key Bcl-2 family proteins. As shown in Fig. 2B, SC-2001 reduced the protein levels of Mcl-1 and Bcl-xl significantly whereas obatoclax had no effect on protein levels of these Bcl-2 family proteins. We then analyzed whether SC-2001 affected the transcription of Mcl-1 in HCC cells. Our data showed that SC-2001 significantly decreased the transcription of Mcl-1 in HepG2 and PLC5 in a time-dependent manner (Fig. 2C and D). These data suggest that SC-2001 reduced protein levels of Mcl-1 through inhibition of transcription.

3.3. Downregulation of p-STAT3 contributes to the apoptotic effect of SC-2001 in HCC

To elucidate the underlying mechanism by which SC-2001 reduced Mcl-1 transcription, we hypothesized that STAT3 may play a role in mediating the effects of SC-2001 on Mcl-1. As shown in Fig. 3A, SC-2001, but not obatoclax, downregulated p-STAT3 at tyrosine 705 in a dose- and time-dependent manner (Fig. 3A). Notably, total STAT3 protein was not affected by SC-2001 in a dose-escalation assay. The phosphorylation of STAT3 at tyrosine residue 705 activated STAT3 and triggered subsequent activation of proteins participating in cell survival and proliferation. Consequently, the downstream effectors driven by STAT3, such as cyclin D1, and survivin, were also repressed by SC-2001 in HCC cells (Fig. 3B). Notably, SC-2001 suppressed interleukin 6 (IL-6)-induced STAT3 activation (Fig. 3C). To further validate the role of STAT3 in SC-2001-induced apoptosis in HCC, we next generated HCC cells with stable expression of STAT3. As shown in Fig. 3D, ectopic expression of STAT3 reversed downregulation of p-STAT3 and abolished the apoptotic effect of SC-2001 in PLC5 cells, indicating that STAT3 mediates SC-2001-induced apoptosis in HCC.

3.4. SHP-1 mediates effects of SC-2001 on p-STAT3

To further study the mechanism by which SC-2001 downregulates p-STAT3 in HCC cells, we examined the role of several protein phosphatases that may participate in SC-2001-induced p-STAT3 inhibition. First, we assessed whether sodium vanadate, a non-specific phosphatase inhibitor, was associated with SC-2001-induced p-STAT3 inhibition. Sodium vanadate abolished the effects of SC-2001 on p-STAT3 and apoptosis, suggesting that protein phosphatases may play a role in mediating the effects of SC-2001 on p-STAT3 (Fig. 4A). Several phosphatases have been reported to be associated with dephosphorylation of STAT3, including SHP-1, SHP-2, and PTP-1B. We next examined the protein levels of these phosphatases in SC-2001-treated HCC cells. Our data indicated that SC-2001 upregulated the protein level of SHP-1 in all three HCC cell lines whereas SC-2001 had no effects on SHP-2 and PTP-1B (Fig. 4B, *top*). SC-2001 increased the transcription of SHP-1 in HCC cells in a dose-dependent or time-dependent manner (Fig. 4B, *bottom*). We then employed a specific SHP-1 inhibitor and found that it significantly reduced SC-2001-induced downregulation of p-STAT3 and apoptosis (Fig. 4C, *left*). In addition, silencing SHP-1 with small-interference RNA (siRNA) abolished the effects of SC-2001 on p-STAT3 (Fig. 4C, *right*). To understand the effect of SC-2001 on SHP-1, we further examined the phosphatase activity of SHP-1 in SC-2001-treated HCC cells. Three cells were exposed to SC-2001 and SHP-1 inhibitor at 10 μM for 6 h and then cell lysates were collected for detection of SHP-1 activity. SC-2001 increased SHP-1 activity significantly in the three HCC cell lines tested (Fig. 4D). These data indicate that SHP-1 is indispensible in mediating the effect of SC-2001 on p-STAT3 and apoptosis in HCC.

3.5. Effects of SC-2001 in PLC5 xenograft nude mice

To assay whether the biological effect of SC-2001 in HCC cells is potentially clinically relevant, we tested the *in vivo* effect of SC-2001 on tumor growth in HCC-bearing xenograft mice. PLC5-bearing mice were treated with vehicle (1X phosphate buffered saline, PBS) or SC-2001 p.o. at 10 mg/kg/every other day for three weeks. As shown in Fig. 5A, SC-2001 significantly inhibited the growth of PLC5 xenograft tumors (Fig. 5A). In addition, analysis of tumor weight showed a significant difference between the control and treatment groups (data not shown). On the contrary, obatoclax did not significantly inhibit the growth of PLC5 xenograft tumors (Fig. S1). To further understand the underlying mechanism by which SC-2001 inhibited tumor growth *in vivo*, we analyzed levels of expression of p-STAT3, SHP-1 and phosphatase activity of SHP-1 in tumor samples. We found that SC-2001 downregulated p-STAT3 in PLC5 tumors (Fig. 5B). Moreover, we verified SHP-1 activity and expression in SC-2001-treated PLC5 tumors. Treatment with SC-2001 significantly increased SHP-1 activity in PLC5 tumors

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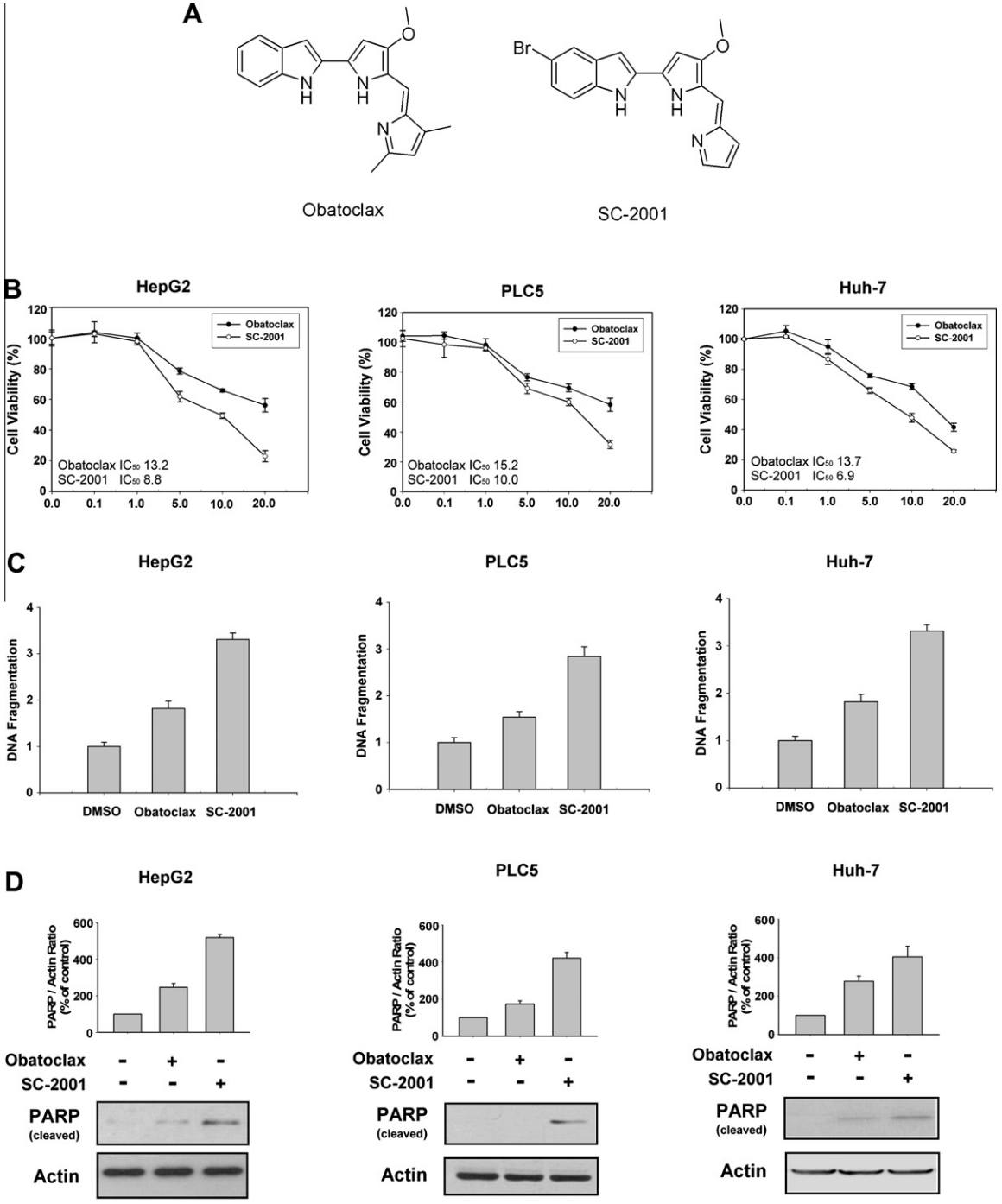

Fig. 1. SC-2001 shows growth-inhibitory effects in HCC cell lines. A, chemical structure of obatoclax and SC-2001. B, dose-escalation effects of obatoclax and SC-2001 on cell viability in three HCC cell lines. Cells were exposed to obatoclax or SC-2001 at the indicated doses for 48 h and cell viability was assessed by MTT assay. *Points*, mean; *bars*, SD ($n = 8$). C, effects of obatoclax and SC-2001 on apoptosis in HCC cells. Cells were exposed to obatoclax or SC-2001 at 10 μM for 12 h. DNA fragmentation was measured by cell death detection ELISA. *Points*, mean; *bars*, SD ($n = 6$). D, effects of obatoclax and SC-2001 on PARP cleavage. Cells were exposed to obatoclax or SC-2001 at 10 μM for 24 h.

(Fig. 5C). Together, these data indicate that SC-2001 inhibited tumor growth through SHP-1-mediated STAT3 inactivation.

4. Discussion

In this study, we discovered that SC-2001 has potent anti-tumor activity that is induced by a novel drug mechanism (SHP-1-dependent STAT3 inactivation) that is distinct from that of obatoclax, and that this important mechanism is responsible for SC-2001 induced cell death in HCC cells. Our data have several important implications: First, our results strengthen the evidence that STAT3 may be a promising anti-cancer target in HCC therapy. We found that obatoclax showed some anti-HCC activity (Fig. 1B and C) probably through Mcl-1 inhibition, whereas SC-2001 showed additional p-STAT3 inhibition. We confirmed that p-STAT3 is the predominant mediator of SC-2001 induced apoptosis (Fig. 3). Currently there are no known oncogene addictions[28] specific and critical to HCC progression [29]. In fact HCC is well-known for its genetic

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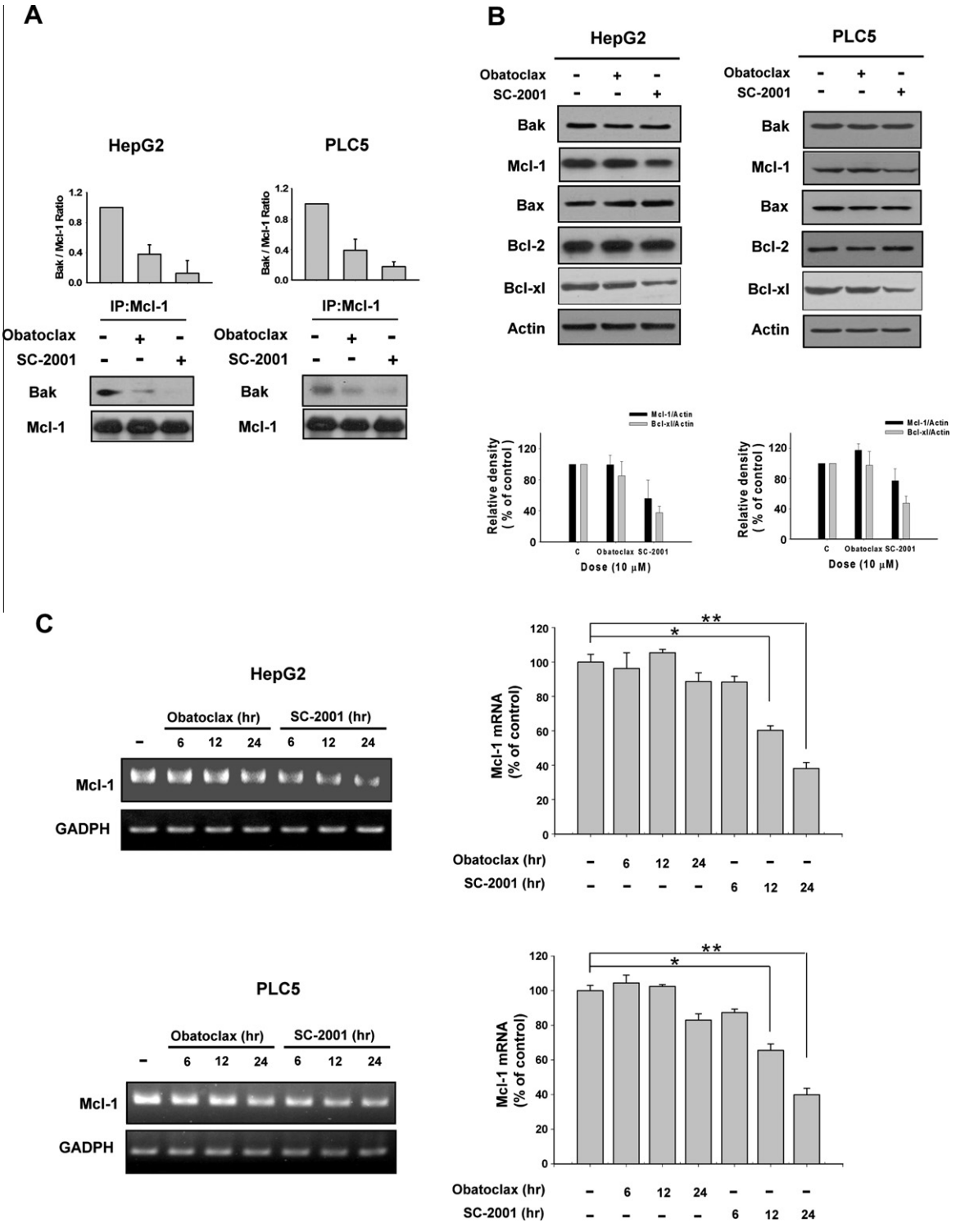

Fig. 2. SC-2001 downregulates Mcl-1 transcription. A, effects of obatoclax and SC-2001 on protein–protein interactions between Mcl-1 and Bak in 2 HCC cell lines. Cells were treated with obatoclax or SC-2001 at 10 μM for 6 h. B, effects of obatoclax and SC-2001 on Bcl-2 family proteins. Cells were treated with obatoclax or SC-2001 at 10 μM for 12 h. Bak, Mcl-1, Bax, Bcl-2 and Bcl-xl were examined by western blot. C, *left*, time-dependent effect of SC-2001 on mRNA of Mcl-1 in HepG2 cells. Cells were exposed to obatoclax or SC-2001 at 10 μM for the indicated intervals. *Right,* the ratio of Mcl-1 to GADPH. *Columns*, mean; *bars*, SD ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$. D, *left*, time-dependent effect of SC-2001 on mRNA of Mcl-1 in PLC5 cells. Cells were exposed to obatoclax or SC-2001 at 10 μM for the indicated intervals. *Right,* the ratio of Mcl-1 to GADPH. *Columns*, mean; *bars*, SD ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$.

heterogeneity and various aberrant signaling cascades, such as EGFR, Ras/raf/ERK, PI3 K/mTOR, hepatocyte growth factor/mesenchymal-epithelial transition factor (HGF/MET), Wnt, Hedgehog, and apoptotic signaling [29,30]. Recently, constitutively activated JAK/STAT signaling has emerged as the dominant mediator in the oncogenesis of HCC. For example, loss of or suppressed function of SOCS family members which are known negative regulators of JAK/STAT signaling, such as aberrant epigenetic silencing of SOCS1

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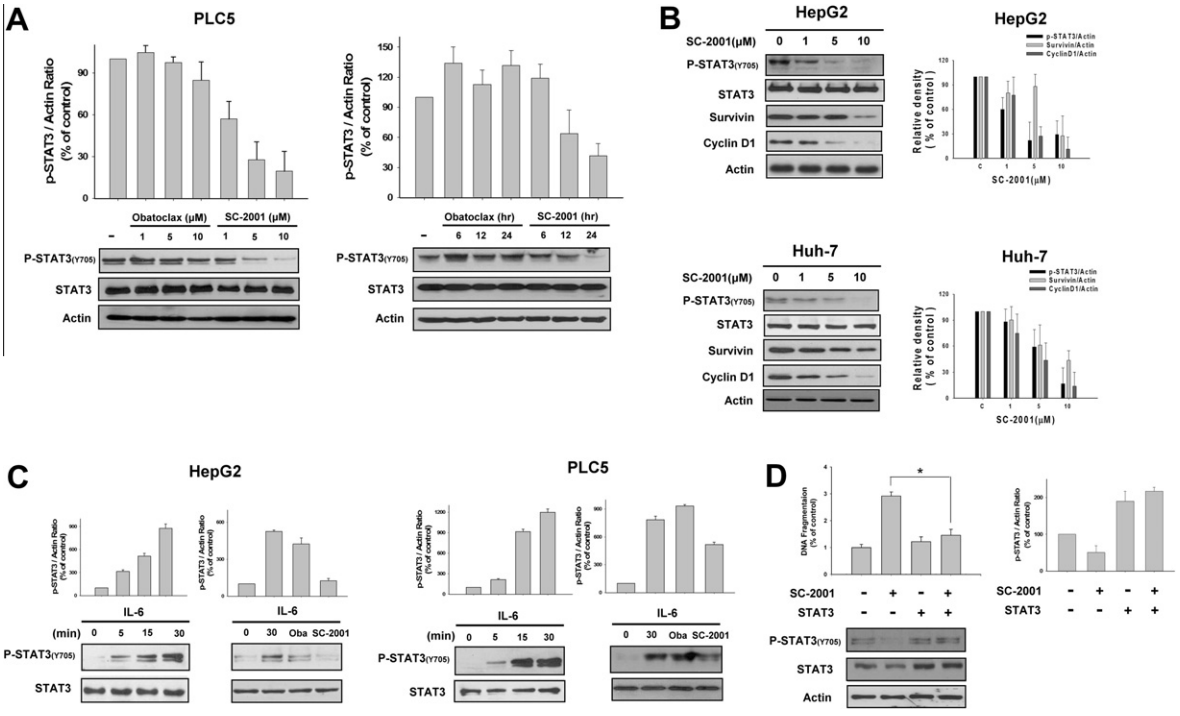

Fig. 3. Downregulation of p-STAT3 contributes to the apoptotic effect of SC-2001 on HCC. A, *top*, dose-dependent effect of SC-2001 on p-STAT3 in PLC5 cells. Cells were treated with SC-2001 or obatoclax at the indicated dose for 12 h. *Bottom,* time-dependent effect of SC-2001 on p-STAT3 in PLC5 cells. Cells were treated with SC-2001 or obatoclax at 10 μM for the indicated time. B, effects of SC-2001 on STAT3-related proteins in HepG2 and Huh-7 cell lines. Cells were exposed to SC-2001 at the indicated doses for 24 h. C, effects of SC-2001 on IL-6-induced STAT3 activation in HepG2 and PLC5 cells. Cells were exposed to SC-2001 at 10 μM for the indicated intervals. D, protective effects of STAT3 on SC-2001-induced apoptosis in PLC5 cells. Cells (wild-type or ectopic expression of STAT3) were exposed to SC-2001 at 10 μM for 12 h. Apoptotic effect was analyzed by cell death ELISA. *Columns*, mean; *bars*, SD ($n = 6$). $^{*}P < 0.05$.

or SOCS-3, [31,32], have been shown to be crucial events in the development of HCC. The JAK/STAT pathway, activated concomitantly with PI3 K/Akt and ERK signaling, has been shown to be crucial mediator of HCC aggressiveness (tumor growth, invasiveness and migration) [33]. Moreover, STAT3 activity in tumor cells may drive the immune response in cancer towards tumor progression [34]. Yu et al. showed that STAT3 activity in tumor cells promotes the synthesis of cytokines (IL-6, IL-10) and growth factors (VEGF) that impairs the maturation of dendritic cells and compromises their ability to stimulate the antitumor effects of CD8 + T cells and natural killer (NK) cells [35]. These findings highlight the fundamental role of STAT3 in carcinogenesis, cell survival and tumor progression in HCC. Importantly, several novel or investigational agents have been shown to have anti-HCC activity through targeting STAT3 [18,20,36,37]. Taken together, these data suggest that STAT3 is a feasible target for the development of novel anti-cancer agents for HCC treatment.

Second, the finding that SC-2001 acts as a SHP-1 dependent p-STAT3 inhibitor lends credence to a new HCC drug discovery strategy, i.e., targeting the interactions of protein tyrosine phosphatases and tyrosine kinases. Among various strategies for developing drugs targeting p-STAT3 [16,38], this phosphatase-directed approach for anti-HCC treatment represents a relatively new proof of concept. Previously we found that sorafenib also inhibits p-STAT3 through enhancing SHP-1 [20,26]. Sorafenib increases SHP-1 activity but does not increase SHP-1 expression, whereas SC-2001 enhances the transcription of SHP-1 mRNA. Interestingly, other compounds such as β-escin and gamma-tocotrienol have been shown to induce expression of SHP-1 that correlates with the downregulation of constitutive STAT3 activation and their anti-HCC activity [36,39]. Similarly, acety-11-keto-beta-boswellic acid, butein (3,4,2′,4′-tetrahydroxychalcone) and betulinic acid have also been shown to induce SHP-1 expression that inhibits p-STAT3, and correlates with their apoptotic and anti-proliferative effects on multiple myeloma cells [23,24]. Taken together, these structurally unrelated agents show a common mechanism, suggesting that targeting the interactions of phosphatases and oncokinases could be a novel anti-HCC or anti-cancer strategy.

Despite the current results, the detailed mechanism by which SC-2001 enhances the transcription of SHP-1 remains unknown. Structurally, obatoclax is a synthetic indole bipyrrol derivative and SC-2001 differs from obatoclax at the 5-position of indole structure, where hydrogen is substituted by bromine. This replacement of hydrogen with bromine theoretically increases the electron density of the aromatic ring thus increasing its strength as an electron donor. On the other hand, the replacement of dimethyl pyrrol by pyrrol reduces the electron donor and steric hinderance effect of the pyrrol ring to the target protein. Like all protein tyrosine phosphatases, SHP-1 has a conserved cysteine residue in its active site essential for its biological activity [40] and can be regulated by redox changes [41]. SC-2001 might have directly increased SHP-1 activity (as shown in Figs. 4D and 5C) through acting as a reducer (electron donor). However, this explanation remains a hypothesis and whether this structural difference contributes directly to the transcription of SHP-1 is unclear, further work is definitely warranted. Moreover, STAT3 activity can be triggered by IL-6 through receptor-associated JAKs; by growth factor RTKs such as the EGFR and VEFGR; or by non-receptor tyrosine kinases like SRC [35]. STAT3 activity is also regulated through a negative feedback mechanism by the SOCS family [42]. In addition, several PTPs have been implicated in STAT3 signaling including SHP-1, SHP-2, PTP-1B [24]. It has been found that loss of SHP-1 may contribute to the activation of JAK or STAT proteins in cancer and SHP-1 has been implicated as a tumor suppressor [43,44]. Nevertheless, we

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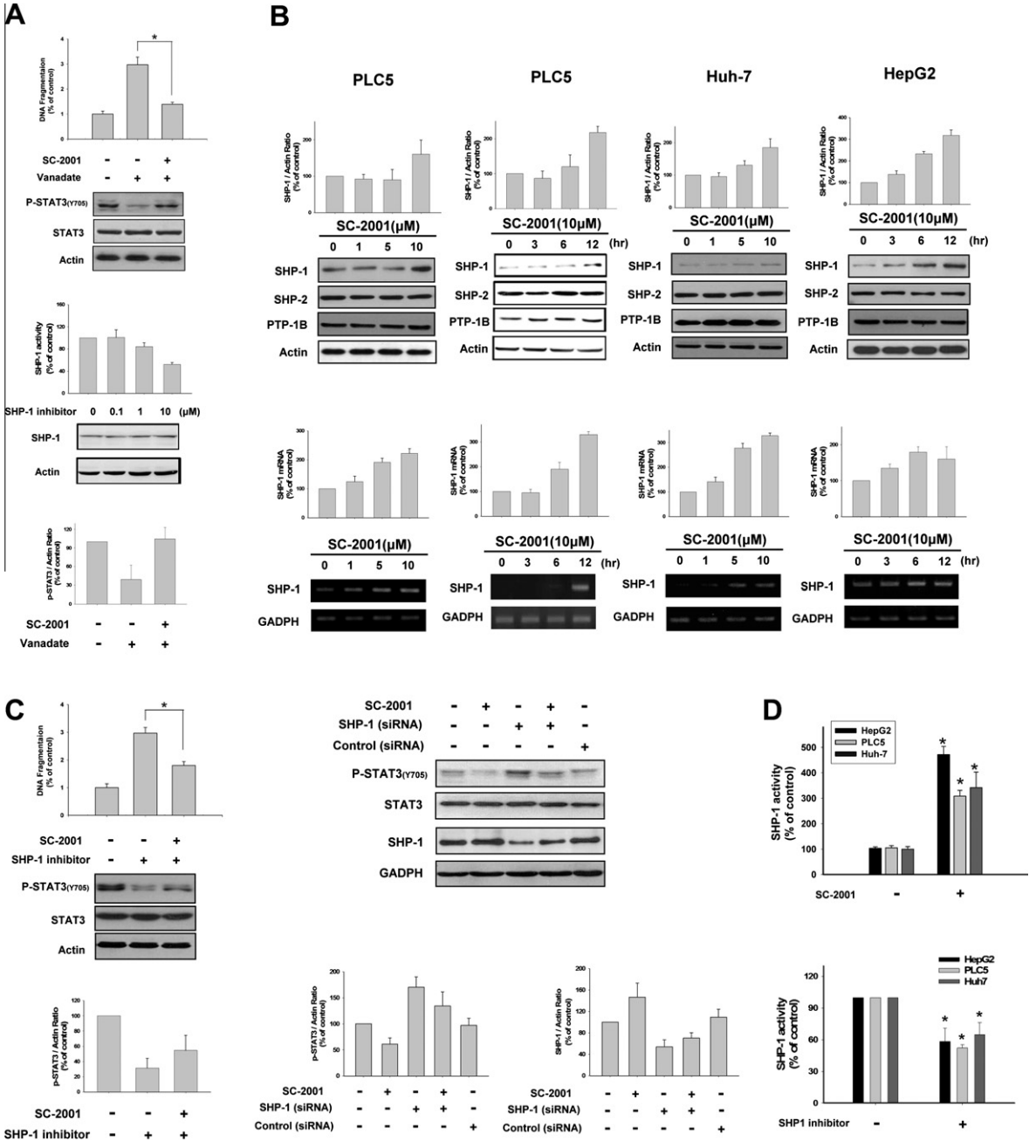

Fig. 4. SHP-1 mediates effects of SC-2001 on p-STAT3. A, sodium vanadate, a non-specific phosphatase inhibitor abolishes downregulation of p-STAT3 and the apoptotic effect of SC-2001 in PLC5 cells. SHP-1 activity is reduced by a specific SHP-1 inhibitor in a dose dependent manner in PLC5 cells. Apoptosis was determined by cell death ELISA. B, SC-2001 increases the protein levels of SHP-1 in all tested HCC cells. *Top,* protein levels. *Bottom,* mRNA. Cells were treated with SC-2001 at the indicated doses for 12 h. In addition, cells were treated with SC-2001 at the indicated time point at 10 μM. C, *left*, a specific SHP-1 inhibitor abolished effects of SC-2001 on p-STAT3 in PLC5 cells. Apoptotic effect was determined by cell death ELISA. *Columns*, mean; *bars*, SD ($n = 6$). $^{*}P < 0.05$. *Middle*, silencing SHP-1 by siRNA reduced the effects of SC-2001 on p-STAT3 in HCC cells. PLC5 cells were transfected with control siRNA or SHP-1 siRNA for 48 h then treated with SC-2001 at 10 μM for 12 h. D, SHP-1 activity in SC-2001-treated and SHP-1 inhibitor-treated HCC cells. Cells were treated with SC-2001 and SHP-1 inhibitor at 10 μM for 6 h and then cell lysates were analyzed by phosphatase activity assay. *Columns*, mean; *bars*, SD ($n = 6$). $^{*}P < 0.05$.

confirmed that the enhancement of SHP-1 activity by SC-2001 correlated with the downregulation of constitutive STAT3 phosphorylation and SC-2001 induced apoptosis. In addition, we examined the effects of SC-2001 on JAK2, Akt and ERK phosphorylation and found that SC-2001 did not significantly alter the phosphorylation of these kinases (Fig. S2). Whether SC-2001 also affects other putative regulators as mentioned above requires further investigation.

In conclusion, SC-2001 is not only a direct Mcl-1 inhibitor but also exhibits promising anti-HCC activity through a novel phosphatase–kinase interactive mechanism, the SHP-1-dependent inhibition of p-STAT3. Our study indicates that the STAT3 signaling pathway may be a suitable target for the development of targeted agents in HCC and that SC-2001 could be further tested for its anticancer activity extending to other cancer types. Future studies defining STAT3 as a useful therapeutic biomarker for HCC patients, as well as the detailed mechanism by which SC-2001 affects SHP-1 activity may lead to further progress in the development of molecular-targeted therapy for HCC.

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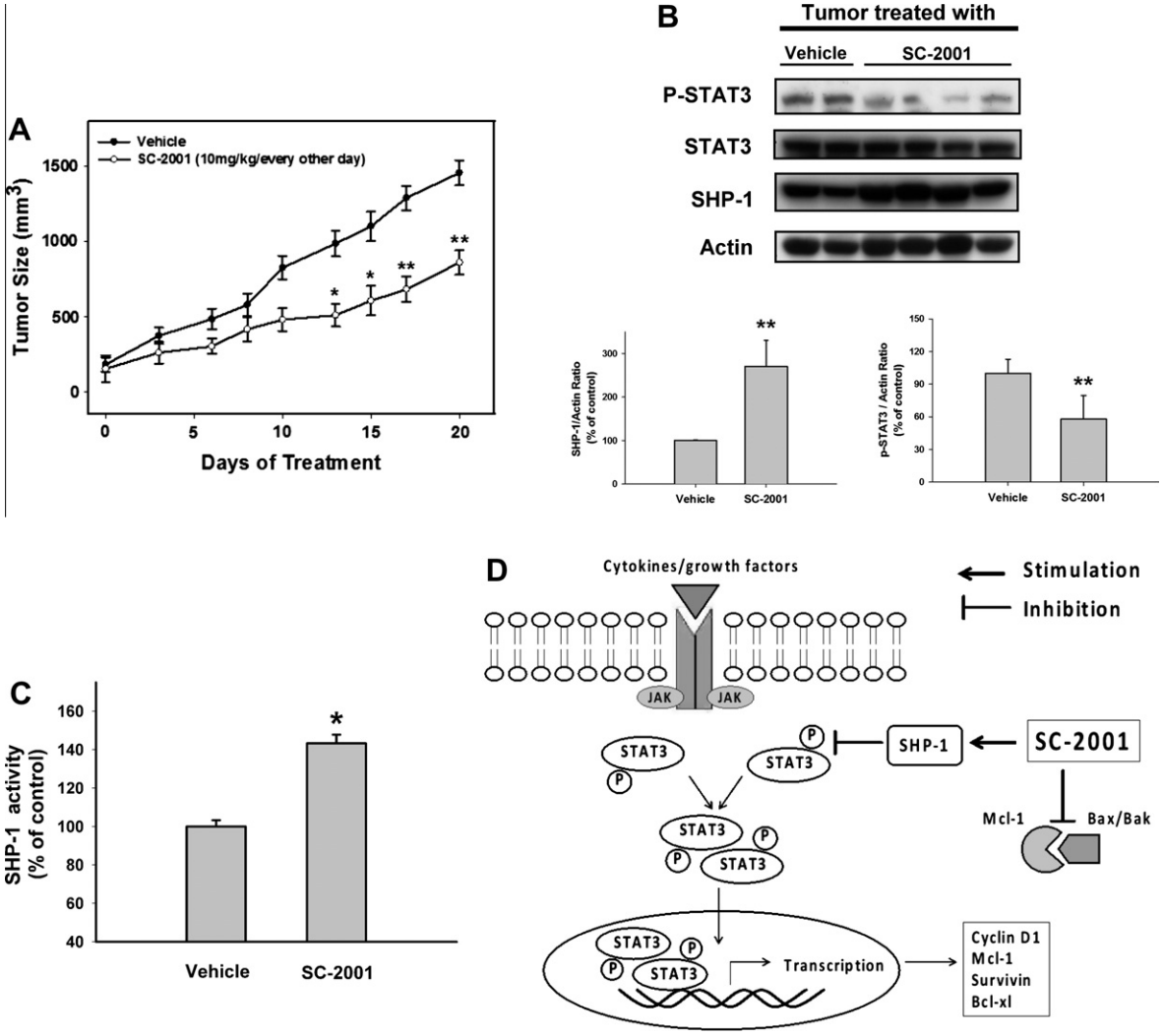

Fig. 5. *In vivo* effects of SC-2001 on PLC5 xenograft nude mice. A, SC-2001 showed significant antitumor effect on PLC5 tumors. *Points*, mean ($n = 10$); *bars*, SE. $^{*}P < 0.05$; $^{**}P < 0.01$. B, analysis of p-STAT3, STAT3 and SHP-1 in PLC5 tumors. C, SHP-1 phosphatase activity in PLC5 tumor. *Columns*, mean; *bars*, SD ($n = 6$). $^{*}P < 0.05$; $^{**}P < 0.01$. D, the drug mechanism of SC-2001 on STAT-3 inhibition and cell apoptosis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.canlet.2012.03.023.

References

[1] C. Verslype, E. Van Cutsem, M. Dicato, N. Arber, J.D. Berlin, D. Cunningham, A. De Gramont, E. Diaz-Rubio, M. Ducreux, T. Gruenberger, D. Haller, K. Haustermans, P. Hoff, D. Kerr, R. Labianca, M. Moore, B. Nordlinger, A. Ohtsu, P. Rougier, W. Scheithauer, H.J. Schmoll, A. Sobrero, J. Tabernero, C. van de Velde, The management of hepatocellular carcinoma. In: Current Expert Opinion and Recommendations Derived from the 10th World Congress on Gastrointestinal Cancer, Barcelona, 2008, Ann. Oncol. 20 (Suppl. 7) (2009) vii1-vii6.

[2] S. Tanaka, S. Arii, Molecularly targeted therapy for hepatocellular carcinoma, Cancer Sci. 100 (2009) 1–8.

[3] J.M. Llovet, S. Ricci, V. Mazzaferro, P. Hilgard, E. Gane, J.F. Blanc, A.C. de Oliveira, A. Santoro, J.L. Raoul, A. Forner, M. Schwartz, C. Porta, S. Zeuzem, L. Bolondi, T.F. Greten, P.R. Galle, J.F. Seitz, I. Borbath, D. Haussinger, T. Giannaris, M. Shan, M. Moscovici, D. Voliotis, J. Bruix, Sorafenib in advanced hepatocellular carcinoma, New Engl. J. Med. 359 (2008) 378–390.

[4] A.L. Cheng, Y.K. Kang, Z. Chen, C.J. Tsao, S. Qin, J.S. Kim, R. Luo, J. Feng, S. Ye, T.S. Yang, J. Xu, Y. Sun, H. Liang, J. Liu, J. Wang, W.Y. Tak, H. Pan, K. Burock, J. Zou, D. Voliotis, Z. Guan, Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial, Lancet Oncol. 10 (2009) 25–34.

[5] J.L. Mott, G.J. Gores, Piercing the armor of hepatobiliary cancer: Bcl-2 homology domain 3 (BH3) mimetics and cell death, Hepatology 46 (2007) 906–911.

[6] I. Marzo, J. Naval, Bcl-2 family members as molecular targets in cancer therapy, Biochem. Pharmacol. 76 (2008) 939–946.

[7] B. Fleischer, H. Schulze-Bergkamen, M. Schuchmann, A. Weber, S. Biesterfeld, M. Muller, P.H. Krammer, P.R. Galle, Mcl-1 is an anti-apoptotic factor for human hepatocellular carcinoma, Int. J. Oncol. 28 (2006) 25–32.

[8] W. Sieghart, D. Losert, S. Strommer, D. Cejka, K. Schmid, S. Rasoul-Rockenschaub, M. Bodingbauer, R. Crevenna, B.P. Monia, M. Peck-Radosavljevic, V. Wacheck, Mcl-1 overexpression in hepatocellular carcinoma: a potential target for antisense therapy, J. Hepatol. 44 (2006) 151–157.

[9] T. Takehara, X. Liu, J. Fujimoto, S.L. Friedman, H. Takahashi, Expression and role of Bcl-xL in human hepatocellular carcinomas, Hepatology 34 (2001) 55–61.

[10] J. Watanabe, F. Kushihata, K. Honda, K. Mominoki, S. Matsuda, N. Kobayashi, Bcl-xL overexpression in human hepatocellular carcinoma, Int. J. Oncol. 21 (2002) 515–519.

[11] Z. Huang, Bcl-2 family proteins as targets for anticancer drug design, Oncogene 19 (2000) 6627–6631.
[12] J.J. Hwang, J. Kuruvilla, D. Mendelson, M.J. Pishvaian, J.F. Deeken, L.L. Siu, M.S. Berger, J. Viallet, J.L. Marshall, Phase I dose finding studies of obatoclax (GX15-070), a small molecule pan-BCL-2 family antagonist, in patients with advanced solid tumors or lymphoma, Clin. Cancer Res. 16 (2010) 4038–4045.
[13] S.M. O'Brien, D.F. Claxton, M. Crump, S. Faderl, T. Kipps, M.J. Keating, J. Viallet, B.D. Cheson, Phase I study of obatoclax mesylate (GX15-070), a small molecule pan-Bcl-2 family antagonist, in patients with advanced chronic lymphocytic leukemia, Blood 113 (2009) 299–305.
[14] P.K. Paik, C.M. Rudin, A. Brown, N.A. Rizvi, N. Takebe, W. Travis, L. James, M.S. Ginsberg, R. Juergens, S. Markus, L. Tyson, S. Subzwari, M.G. Kris, L.M. Krug, A phase I study of obatoclax mesylate, a Bcl-2 antagonist, plus topotecan in solid tumor malignancies, Cancer Chemother. Pharm. 66 (2010) 1079–1085.
[15] A.D. Schimmer, S. O'Brien, H. Kantarjian, J. Brandwein, B.D. Cheson, M.D. Minden, K. Yee, F. Ravandi, F. Giles, A. Schuh, V. Gupta, M. Andreeff, C. Koller, H. Chang, S. Kamel-Reid, M. Berger, J. Viallet, G. Borthakur, A phase I study of the pan bcl-2 family inhibitor obatoclax mesylate in patients with advanced hematologic malignancies, Clin. Cancer Res. 14 (2008) 8295–8301.
[16] D. Germain, D.A. Frank, Targeting the cytoplasmic and nuclear functions of signal transducers and activators of transcription 3 for cancer therapy, Clin. Cancer Res. 13 (2007) 5665–5669.
[17] W.C. Li, S.L. Ye, R.X. Sun, Y.K. Liu, Z.Y. Tang, Y. Kim, J.G. Karras, H. Zhang, Inhibition of growth and metastasis of human hepatocellular carcinoma by antisense oligonucleotide targeting signal transducer and activator of transcription 3, Clin. Cancer Res. 12 (2006) 7140–7148.
[18] M. Kusaba, K. Nakao, T. Goto, D. Nishimura, H. Kawashimo, H. Shibata, Y. Motoyoshi, N. Taura, T. Ichikawa, K. Hamasaki, K. Eguchi, Abrogation of constitutive STAT3 activity sensitizes human hepatoma cells to TRAIL-mediated apoptosis, J. Hepatol. 47 (2007) 546–555.
[19] K.F. Chen, W.T. Tai, J.W. Huang, C.Y. Hsu, W.L. Chen, A.L. Cheng, P.J. Chen, C.W. Shiau, Sorafenib derivatives induce apoptosis through inhibition of STAT3 independent of Raf, Eur. J. Med. Chem. 46 (2011) 2845–2851.
[20] K.F. Chen, W.T. Tai, T.H. Liu, H.P. Huang, Y.C. Lin, C.W. Shiau, P.K. Li, P.J. Chen, A.L. Cheng, Sorafenib overcomes TRAIL resistance of hepatocellular carcinoma cells through the inhibition of STAT3, Clin. Cancer Res. 16 (2010) 5189–5199.
[21] Y. Han, H.M. Amin, B. Franko, C. Frantz, X. Shi, R. Lai, Loss of SHP1 enhances JAK3/STAT3 signaling and decreases proteosome degradation of JAK3 and NPM-ALK in ALK+ anaplastic large-cell lymphoma, Blood 108 (2006) 2796–2803.
[22] A. Witkiewicz, P. Raghunath, A. Wasik, J.M. Junkins-Hopkins, D. Jones, Q. Zhang, N. Odum, M.A. Wasik, Loss of SHP-1 tyrosine phosphatase expression correlates with the advanced stages of cutaneous T-cell lymphoma, Hum. Pathol. 38 (2007) 462–467.
[23] M.K. Pandey, B. Sung, B.B. Aggarwal, Betulinic acid suppresses STAT3 activation pathway through induction of protein tyrosine phosphatase SHP-1 in human multiple myeloma cells, Int. J. Cancer 127 (2010) 282–292.
[24] A.B. Kunnumakkara, A.S. Nair, B. Sung, M.K. Pandey, B.B. Aggarwal, Boswellic acid blocks signal transducers and activators of transcription 3 signaling, proliferation, and survival of multiple myeloma via the protein tyrosine phosphatase SHP-1, Mol. Cancer Res. 7 (2009) 118–128.
[25] S.K. Sandur, M.K. Pandey, B. Sung, B.B. Aggarwal, 5-hydroxy-2-methyl-1,4-naphthoquinone, a vitamin K3 analogue, suppresses STAT3 activation pathway through induction of protein tyrosine phosphatase, SHP-1: potential role in chemosensitization, Mol. Cancer Res. 8 (2010) 107–118.
[26] W.T. Tai, A.L. Cheng, C.W. Shiau, H.P. Huang, J.W. Huang, P.J. Chen, K.F. Chen, Signal transducer and activator of transcription 3 is a major kinase-independent target of sorafenib in hepatocellular carcinoma, J. Hepatol. 55 (2011) 1041–1048.
[27] K.F. Chen, P.Y. Yeh, C. Hsu, C.H. Hsu, Y.S. Lu, H.P. Hsieh, P.J. Chen, A.L. Cheng, Bortezomib overcomes tumor necrosis factor-related apoptosis-inducing ligand resistance in hepatocellular carcinoma cells in part through the inhibition of the phosphatidylinositol 3-kinase/Akt pathway, J. Biol. Chem. 284 (2009) 11121–11133.
[28] I.B. Weinstein, A. Joe, Oncogene addiction, Cancer Res. 68 (2008) 3077–3080; discussion 3080.
[29] A. Villanueva, J.M. Llovet, Targeted therapies for hepatocellular carcinoma, Gastroenterology 140 (2011) 1410–1426.
[30] J.M. Llovet, J. Bruix, Molecular targeted therapies in hepatocellular carcinoma, Hepatology 48 (2008) 1312–1327.
[31] T.V. Bagnyukova, V.P. Tryndyak, L. Muskhelishvili, S.A. Ross, F.A. Beland, I.P. Pogribny, Epigenetic downregulation of the suppressor of cytokine signaling 1 (Socs1) gene is associated with the STAT3 activation and development of hepatocellular carcinoma induced by methyl-deficiency in rats, Cell Cycle 7 (2008) 3202–3210.
[32] H. Ogata, T. Kobayashi, T. Chinen, H. Takaki, T. Sanada, Y. Minoda, K. Koga, G. Takaesu, Y. Maehara, M. Iida, A. Yoshimura, Deletion of the SOCS3 gene in liver parenchymal cells promotes hepatitis-induced hepatocarcinogenesis, Gastroenterology 131 (2006) 179–193.
[33] N.K. Saxena, D. Sharma, X. Ding, S. Lin, F. Marra, D. Merlin, F.A. Anania, Concomitant activation of the JAK/STAT, PI3K/AKT, and ERK signaling is involved in leptin-mediated promotion of invasion and migration of hepatocellular carcinoma cells, Cancer Res. 67 (2007) 2497–2507.
[34] C. Berasain, J. Castillo, M.J. Perugorria, M.U. Latasa, J. Prieto, M.A. Avila, Inflammation and liver cancer: new molecular links, Ann. NY Acad. Sci. 1155 (2009) 206–221.
[35] H. Yu, M. Kortylewski, D. Pardoll, Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment, Nat. Rev. Immunol. 7 (2007) 41–51.
[36] P. Rajendran, F. Li, K.A. Manu, M.K. Shanmugam, S.Y. Loo, A.P. Kumar, G. Sethi, Gamma-Tocotrienol is a novel inhibitor of constitutive and inducible STAT3 signalling pathway in human hepatocellular carcinoma: potential role as an antiproliferative, pro-apoptotic and chemosensitizing agent, Brit. J. Pharmacol. 163 (2011) 283–298.
[37] P. Rajendran, T.H. Ong, L. Chen, F. Li, M.K. Shanmugam, S. Vali, T. Abbasi, S. Kapoor, A. Sharma, A.P. Kumar, K.M. Hui, G. Sethi, Suppression of signal transducer and activator of transcription 3 activation by butein inhibits growth of human hepatocellular carcinoma in vivo, Clin. Cancer Res. 17 (2011) 1425–1439.
[38] S. Haftchenary, M. Avadisian, P.T. Gunning, Inhibiting aberrant Stat3 function with molecular therapeutics: a progress report, Anticancer Drugs 22 (2011) 115–127.
[39] S.M. Tan, F. Li, P. Rajendran, A.P. Kumar, K.M. Hui, G. Sethi, Identification of beta-escin as a novel inhibitor of signal transducer and activator of transcription 3/Janus-activated kinase 2 signaling pathway that suppresses proliferation and induces apoptosis in human hepatocellular carcinoma cells, J. Pharmacol. Exp. Ther. 334 (2010) 285–293.
[40] E.H. Fischer, H. Charbonneau, N.K. Tonks, Protein tyrosine phosphatases: a diverse family of intracellular and transmembrane enzymes, Science 253 (1991) 401–406.
[41] F. Esposito, R. Ammendola, R. Faraonio, T. Russo, F. Cimino, Redox control of signal transduction, gene expression and cellular senescence, Neurochem. Res. 29 (2004) 617–628.
[42] L. Larsen, C. Ropke, Suppressors of cytokine signalling: SOCS, APMIS 110 (2002) 833–844.
[43] C. Wu, M. Sun, L. Liu, G.W. Zhou, The function of the protein tyrosine phosphatase SHP-1 in cancer, Gene 306 (2003) 1–12.
[44] C. Wu, Q. Guan, Y. Wang, Z.J. Zhao, G.W. Zhou, SHP-1 suppresses cancer cell growth by promoting degradation of JAK kinases, J. Cell Biochem. 90 (2003) 1026–1037.

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