Cancer Letters 524 (2022) 161–171

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Induction of IL-6Rα by ATF3 enhances IL-6 mediated sorafenib and regorafenib resistance in hepatocellular carcinoma

Zichan Dai [a,1], Xiaohan Wang [a,1], Rangxin Peng [a,1], Binghui Zhang [a], Qi Han [a], Jie Lin [b], Jichuang Wang [a], Junjin Lin [c], Mingting Jiang [a], Hekun Liu [a], Tae Ho Lee [a], Kun Ping Lu [a], Min Zheng [a,d,*]

[a] Fujian Key Laboratory of Translational Research in Cancer and Neurodegenerative Diseases, Institute for Translational Medicine, The School of Basic Medical Sciences, Fujian Medical University, Fujian, PR China
[b] Shengli Clinical Medical College, Fujian Medical University & Department of Pathology, Fujian Provincial Hospital, Fujian, PR China
[c] Public Technology Service Center, Fujian Medical University, Fujian, PR China
[d] Department of Biochemistry and Molecular Biology, The School of Basic Medical Sciences, Fujian Medical University, Fujian, PR China

ARTICLE INFO

Keywords:
IL-6Rα
ATF3
HCC
Sorafenib
Regorafenib

ABSTRACT

Sorafenib and its derivative regorafenib are the first- and second-line targeted drugs for advanced HCC, respectively. Although both drugs improve overall survival, drug resistance remains the major barrier to their full efficacy. Thus, strategies to enhance sorafenib and regorafenib efficacy against HCC are solely needed. Interleukin-6 receptor alpha (IL-6Rα) is the receptor of IL-6, a multi-functional cytokine, which plays key roles in liver-regeneration, inflammation and development of hepatocellular carcinoma (HCC). Here we show the expression of IL-6Rα was induced in response to sorafenib. Depletion of IL-6Rα abolished IL-6 induced STAT3 phosphorylation at 705th tyrosine and tumor growth of HCC cells under sorafenib treatment. Mechanistically, activating transcription factor 3 (ATF3) was induced in response to sorafenib and subsequently bound to the promoter of IL-6Rα, leading to its transcriptional activation. Depletion of ATF3 or its upstream transcription factor, ATF4, attenuated IL-6Rα induction and IL-6 mediated sorafenib resistance. The ATF4-ATF3-IL-6Rα cascade is also activated by regorafenib. Furthermore, blockade of IL-6Rα with the FDA approved IL-6Rα antibody drug, Sarilumab, drastically attenuated both sorafenib and regorafenib resistance in patient-derived xenograft (PDX) tumors, where human IL-6 could be detected by a novel *in situ* hybridization technique, named RNAscope. Together, our data reveal that ATF3-mediated IL-6Rα up-regulation promotes both sorafenib and regorafenib resistance in HCC, and targeting IL-6Rα represents a novel therapeutic strategy to enhance sorafenib/regorafenib efficacy for advanced HCC treatment.

1. Introduction

Hepatocellular carcinoma (HCC) is the fourth major cause of cancer-related deaths worldwide and ranks second in terms of male death rates [1]. Half of HCC patients are diagnosed at an advanced stage where liver resection and transplantation are not feasible [2]. Due to the complex etiology and heterogeneity of HCC, targeted drugs for advanced HCC remain severely limited [3]. From 2007 to 2016, sorafenib, a multi-kinase inhibitor, was the only systemic drug with proven efficacy against advanced HCC [4]. Its derivative, regorafenib, was approved in 2017 as a second-line drug for advanced HCC patients who tolerated sorafenib treatment [5]. However, the overall survival benefits of sorafenib and regorafenib in HCC patients are still limited due to the existence of drug resistance [6,7]. Strategies to overcome drug resistance in HCC are sorely needed.

Interleukin-6 (IL-6) is a potent hepatocyte mitogen and proinflammatory cytokine that promotes liver regeneration and HCC progression [8,9]. Serum IL-6 levels are statistically higher in patients with HCC than in healthy people [10] and increased serum IL-6 levels are associated with an increased risk of HCC [11]; in contrast, IL-6-deficient mice have a lower incidence of DEN-induced HCC [12]. Recently, the

* Corresponding author. Fujian Key Laboratory of Translational Research in Cancer and Neurodegenerative Diseases, Institute for Translational Medicine, The School of Basic Medical Sciences, Fujian Medical University. 1 Xue Yuan Road, University Town, Fuzhou, 350122, PR China.
E-mail address: mzheng@mail.fjmu.edu.cn (M. Zheng).
[1] These authors contributed equally to this paper.

https://doi.org/10.1016/j.canlet.2021.10.024
Received 21 July 2021; Received in revised form 24 September 2021; Accepted 17 October 2021
Available online 20 October 2021

Abbreviations

HCC	hepatocellular carcinoma
DEN	diethylnitrosamine
CDX	cell line derived xenograft
PDX	patient-derived xenograft
IL-6	interleukin-6
IL-6Rα	interleukin-6 receptor alpha
mIL-6Rα	membrane bound interleukin-6 receptor alpha
sIL-6Rα	soluble form of interleukin-6 receptor alpha
ATF3	activating transcription factor 3
ATF4	activating transcription factor 4
JAK	Janus kinase
STAT3	signal transducer and activator of transcription 3
ChIP	chromatin immunoprecipitation
sgRNA	single guide RNA
WT	wild-type
KO	knockout
KD	knockdown
Sora	sorafenib
Rego	regorafenib
Sari	Sarilumab
Toci	Tocilizumab
qRT-PCR	quantitative real-time PCR
bp	base pairs
ELISA	enzyme linked immunosorbent assay
IHC	immunohistochemistry
α-SMA	α-smooth muscle actin
PERK	PKR-Like endoplasmic reticulum kinase

role of IL-6 in chemotherapeutic resistance has attracted more and more attention. IL-6 has been shown to augment chemotherapeutic resistance in lung cancer, gastric cancer and neuroblastoma [13–15]. Likewise, decreased IL-6 induces sensitivity of HCC to sorafenib [16]. Moreover, high plasma IL-6 levels are also associated with poor prognosis of sorafenib in patients with advanced HCC [17].

IL-6Rα (also known as gp80, CD126) is the receptor of IL-6 [18]. Interaction of IL-6 and IL-6Rα recruits the membrane-bound IL-6R beta (also known as gp130, IL-6ST), leading to the activation of intracellular signaling pathways, such as JAK/STAT3 [19], which facilitates tumor survival, proliferation and cancer stemness, favoring drug resistance and metastasis in several human cancers [20,21]. IL-6Rα exists not only as a membrane bound form (mIL-6Rα), but also as a soluble form (sIL-6Rα) [19]. The sIL-6Rα could be a product of alternative splicing variant of IL-6Rα gene, or a proteolytically cleaved product of mIL-6Rα [22,23]. Either mIL-6Rα or sIL-6Rα can bind to IL-6 and recruit gp130 to mediate signal transduction through classical- and *trans*-signaling pathways, respectively [24]. Combined IL-6 and sIL-6Rα accelerates liver regeneration [25]; while blockade of IL-6 *trans*-signaling suppresses HCC development in mice [26], corroborating the pivotal role of IL-6/IL-6Rα signaling in HCC development. However, the expression and role of IL-6Rα in drug resistance of HCC are still largely unknown.

In this study, we uncovered that IL-6Rα was induced by activating transcription factor 4 (ATF4)-ATF3 cascade in response to sorafenib or regorafenib treatment. Importantly, genetic depletion or antibody-based inhibition of IL-6Rα attenuated IL-6 mediated sorafenib and regorafenib resistance both *ex vivo* and *in vivo*. Our findings provide not only the rationale but also the first preclinical evidence that blockade of IL-6Rα potently improves sorafenib and regorafenib efficacy in HCC treatment.

2. Materials and methods

2.1. Antibodies and reagents

Sorafenib (#HY-10201) and regorafenib (#HY-10331) were purchased from MedchemExpress (Shanghai, China). An anti-ATF4 (#10835-1-AP) antibody was purchased from Proteintech (Wuhan, China). Anti-ATF3 (#HPA001562) and anti-Flag (#F1804) antibodies were purchased from Sigma (St. Louis, MO). Anti-eIF2α (#5324), anti-phospho-eIF2α (Ser51) (#3398), anti-STAT3 (#9139), anti-PERK (#3192) and anti-phospho-STAT3 (Tyr705) (#9145) antibodies were from Cell Signaling (Danvers, MA). An anti-α-SMA (ab230458) antibody was from Abcam. Anti-GAPDH (#HC301), anti-β-tubulin (#HC101) and anti-β-Actin (#HC201) antibodies were obtained from Transgen (Beijing, China). The ELISA kit for IL-6Rα was purchased from Proteintech (#KE00118) (Wuhan, China). Protease inhibitor cocktail (#HY-K0011), GSK2606414 (#HY-18072) and phosphatase inhibitors (#HY-K0022) were obtained from MedchemExpress (Shanghai, China). Tocilizumab (#A2012) and Sarilumab (#A2011) were from SelleckChem. IL-6 (#206-IL) was purchased from R&D Systems. Primers for quantitative real-time PCR, RNA interference and sgRNAs are listed in the Supplementary Information.

2.2. ELISA measurement

Cells were treated with drugs for 36 h. The soluble form of IL-6Rα in cell culture supernatants was measured with a human IL-6Rα ELISA kit according to the manufacturer's instructions. Plates were measured on a microplate reader (Thermo Scientific Multiskan GO) at a wavelength of 450 nm.

2.3. Animal models

All animal protocols were approved by the Experimental Animal Ethics Committee of Fujian Medical University according to the Association for Assessment and Accreditation of Laboratory Animal Care International Regulations. BALB/c nude mice were maintained in the specific-pathogen-free (SPF) Laboratory Animal Center of Fujian Medical University. For the patient-derived tumor xenograft (PDX) model, the patient samples derived from a woman aged 67 with HBV positive and low-grade HCC. Patient tumors were cut into similar sizes (2 mm × 2 mm) and inoculated into the right flank of 5-week-old male BALB/c nude mice. For cell line derived xenograft (CDX) tumor models, 1×10^6 HCC cell lines (Huh7 or IL-6Rα-KO Huh7) were resuspended in 100 μL PBS and subcutaneously injected into right flank of 6 weeks old male BALB/c nude mice. Tumor volume was calculated using the following formula: volume (mm^3) = length × width × width × 0.5. Drug administration began when the length of tumors reached ~5 mm and the mice were randomized for treatment as indicated. The protocol has been approved by the Ethical Committee of Fujian Medical University. And the patient provided informed consent.

2.4. RNAscope assay

RNA *in situ* hybridization for human IL-6 mRNA was performed using an RNAscope® 2.5 HD Reagent Kit-BROWN (Advanced Cell Diagnostics, 322300) according to the manufacturer's instructions. In brief, 5-μm-thick formalin-fixed, paraffin-embedded tissue sections were deparaffinized and pre-treated with heat and protease before hybridization with the human IL-6 oligonucleotide probes (Advanced Cell Diagnostics, 310371). The signal is amplified using a multi-step process, followed by hybridization to horseradish peroxidase (HRP)-labeled probes and detection using a chromogenic substrate. The RNA integrity quality was controlled with an RNAscope® positive Control Probe-Hs-PPIB

(Advanced Cell Diagnostics, 313901), and for the background with an RNAscope® negative Control Probe-dapB (Advanced Cell Diagnostics, 310043).

2.5. Statistical analysis

The data are expressed as the means ± (standard error of the mean) of more than three independent experiments. Unpaired Student's *t* tests were used to compare the means of two groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. IL-6Rα is induced in response to sorafenib treatment

Consistent with the previous finding that IL-6 is a potent mitogen for hepatocytes and HCC cells [8], IL-6 enhanced the colony formation of HCC cells in the presence of sorafenib (Fig. 1a), supporting the notion that IL-6 mediates sorafenib resistance in HCC. Interestingly, co-treatment of 5 μM sorafenib for 24 h dramatically increased IL-6 induced STAT3 phosphorylation at tyrosine-705, indicating IL-6 signaling was enhanced in the presence of sorafenib treatment (Fig. 1b). To investigate the underlying mechanism, we performed transcriptome sequencing after human HCC Huh7 cells were treated with 5 μM sorafenib, 10 ng/mL IL-6 or their combination for 12 h. Intriguingly, IL-6Rα, the receptor of IL-6, was dramatically induced by sorafenib (Fig. 1c). To confirm this phenomenon, a second transcriptome sequencing analysis was performed in Huh7 cells treated with 5 μM or 10 μM sorafenib for 24 h. Using a fold change cut-off of >8 and basal FPKM value in untreated cells cut-off of >1, 8 genes including IL-6Rα were found to be highly induced in response to sorafenib treatment (Fig. 1d). Quantitative real-time PCR (qRT-PCR) and ELISA analysis verified IL-6Rα up-regulation by sorafenib in HCC cells (Fig. 1e and f). The role of IL-6Rα is to mediate IL-6 signaling. Indeed, IL-6Rα overexpression enhanced IL-6-induced STAT3 activation, as detected by STAT3 phosphorylation at tyrosine-705 (Fig. 1g), as shown previously [27]. Together, these data suggest that it might be IL-6Rα induction that underlies the mechanism by which short-term sorafenib treatment synergistically enhances IL-6 induced STAT3 activation.

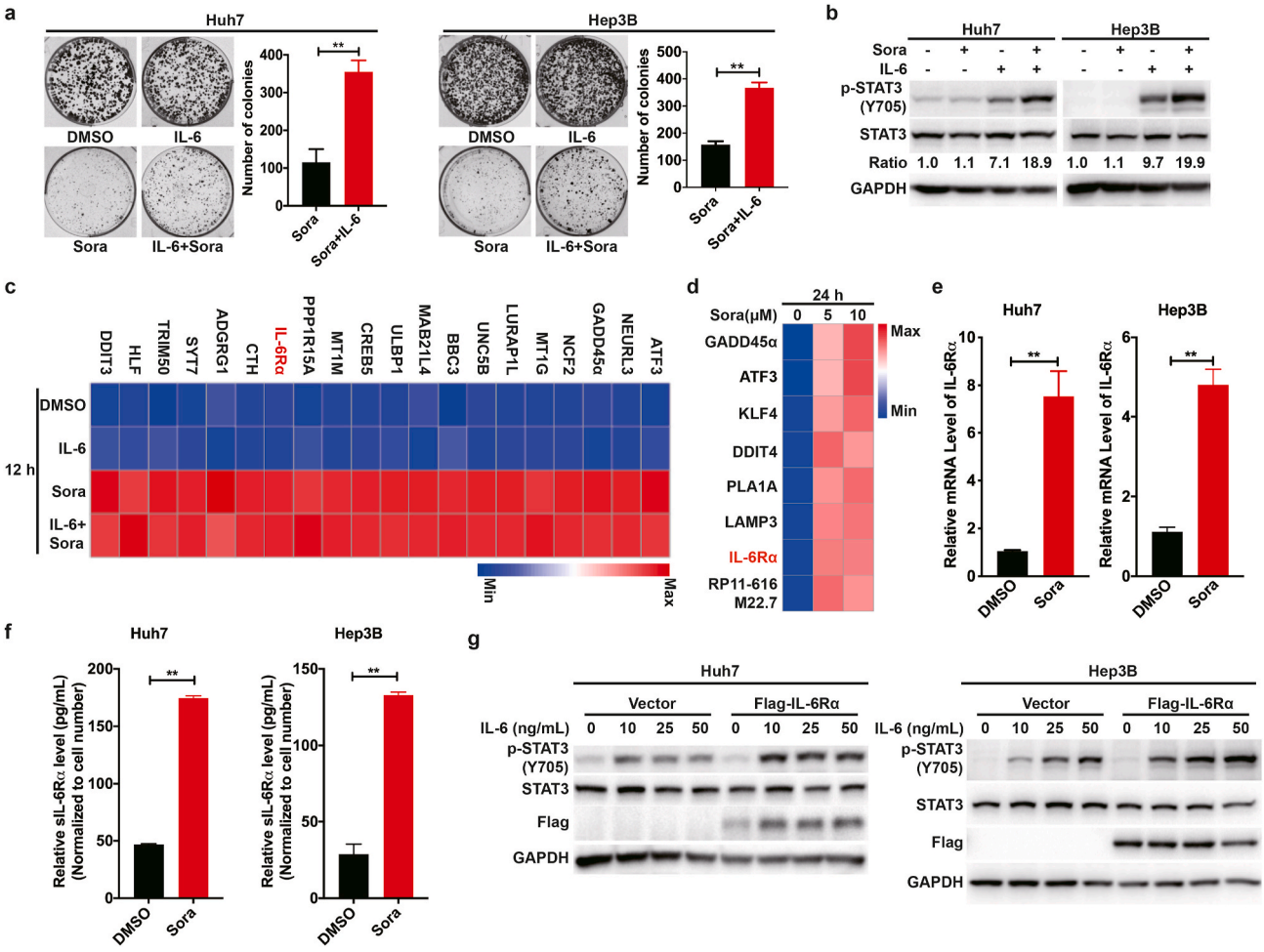

Fig. 1. Sorafenib induces IL-6Rα expression in HCC cells. (a) Human HCC Huh7 and Hep3B cells were treated with DMSO, 5 μM sorafenib, 100 ng/mL IL-6 or their combination. Colony formation assays were performed (left). Quantification of sorafenib and sorafenib + IL-6 clones was performed using Image-Pro Plus software (right). (b) Huh7 and Hep3B cells were treated with 5 μM sorafenib, 10 ng/mL IL-6 or their combination for 24 h. Phosphorylation of STAT3 at tyrosine-705 was determined by immunoblotting. (c) Huh7 cells treated with DMSO, 5 μM sorafenib, 10 ng/mL IL-6 or their combination for 12 h were subjected to transcriptomic analysis using 2nd generation RNA sequencing technique. Using a fold change (the ratio of FPKM value of sorafenib group to DMSO group) cut-off of >4 and basal FPKM value in untreated cells cut-off of >1, the top 20 genes were listed. (d) Huh7 cells treated with DMSO, 5 μM or 10 μM sorafenib for 24 h were subjected to transcriptomic analysis using 2nd generation RNA sequencing technique. (e,f) Huh7 and Hep3B cells were treated with 5 μM sorafenib and subjected to quantitative real-time PCR (e) and ELISA (f) analysis for IL-6Rα. The mRNA levels of IL-6Rα were normalized to those of GAPDH. (g) Huh7 and Hep3B cells were infected with lentiviruses expressing Flag-IL-6Rα and treated with increasing doses of IL-6 for 24 h. STAT3 phosphorylation at tyrosine-705 was determined by immunoblotting. The data are presented as the means ± SEM (*: $P < 0.05$; **: $P < 0.01$; $n = 3$).

3.2. IL-6Rα is essential for IL-6 mediated sorafenib resistance in HCC

Recently, another glycoprotein, CD5, was found to mediate IL-6 signaling in the absence of IL-6Rα [28]. Thus, it would be necessary to investigate whether IL-6Rα is essential for IL-6 mediated STAT3 activation and sorafenib resistance in HCC. We used CRISPR/Cas9 gene editing technique to generate IL-6Rα depleted HCC cells, which were confirmed by ELISA analysis (Fig. 2a). Both IL-6Rα knockout in Huh7 and IL-6Rα knockdown in Hep3B cells abolished the synergistic effect of sorafenib on IL-6 induced STAT3 activation (Fig. 2b). Colony formation assay also showed that IL-6Rα depletion largely attenuated IL-6 mediated sorafenib resistance *ex vivo* (Fig. 2c). To further substantiate these findings, we performed *in vivo* experiments using cell line derived xenograft (CDX) mice model. Intraperitoneal injection of human IL-6 enhanced tumor growth either in the presence or absence of sorafenib treatment, which was abolished by IL-6Rα depletion (Fig. 2d–2f). Thus, IL-6Rα is the only biological relevant receptor for IL-6 in HCC, and is essential for IL-6 mediated sorafenib resistance in HCC.

3.3. ATF3 binds to the IL-6Rα promoter and is involved in IL-6Rα induction

To investigate the mechanism underlying IL-6Rα induction, luciferase assays were performed to screen transcription factors of IL-6Rα in response to sorafenib treatment. Ten transcription factors were selected based on the preconditions that these genes were also induced by sorafenib and had binding sites on IL-6Rα promoter. Interestingly, ATF3 was shown to be the most active transcription factor for IL-6Rα (Supplementary Figs. S1a and S1b). Using the eukaryotic promoter database (EPD) from the Swiss Institute of Bioinformatics [29], an ATF3 binding site at −82 ~ −88 base pairs upstream of the transcriptional start site (TSS) was identified in the IL-6Rα promoter (Supplementary Fig. S1c).

ATF3 binding to this putative promoter region of the IL-6Rα gene was confirmed by chromatin immunoprecipitation (ChIP) assay (Fig. 3a). Deletion of ATF3 binding site largely attenuated the effect of ATF3 on IL-6Rα promoter activity (Fig. 3b and c). These data indicated that ATF3 induces IL-6Rα expression likely via binding to the IL-6Rα promoter. Consistent with the transcriptome sequencing analysis in Fig. 1c and d, sorafenib induced both mRNA and protein expression of ATF3 in both Huh7 and Hep3B cells (Fig. 3d and e). In order to investigate whether ATF3 was involved in sorafenib induced IL-6Rα expression, ATF3-KO Huh7 cells were generated (Fig. 3f). Quantitative real-time PCR analysis in ATF3 depleted Huh7 and their controlled cells treated with or without sorafenib revealed that IL-6Rα mRNA up-regulation was largely suppressed in ATF3-KO Huh7 cell lines (Fig. 3g). ELISA assay also demonstrated that sorafenib induced IL-6Rα in an ATF3 dependent manner (Fig. 3h). Moreover, ATF3 depletion attenuated IL-6 induced STAT3 activation and colony formation in the

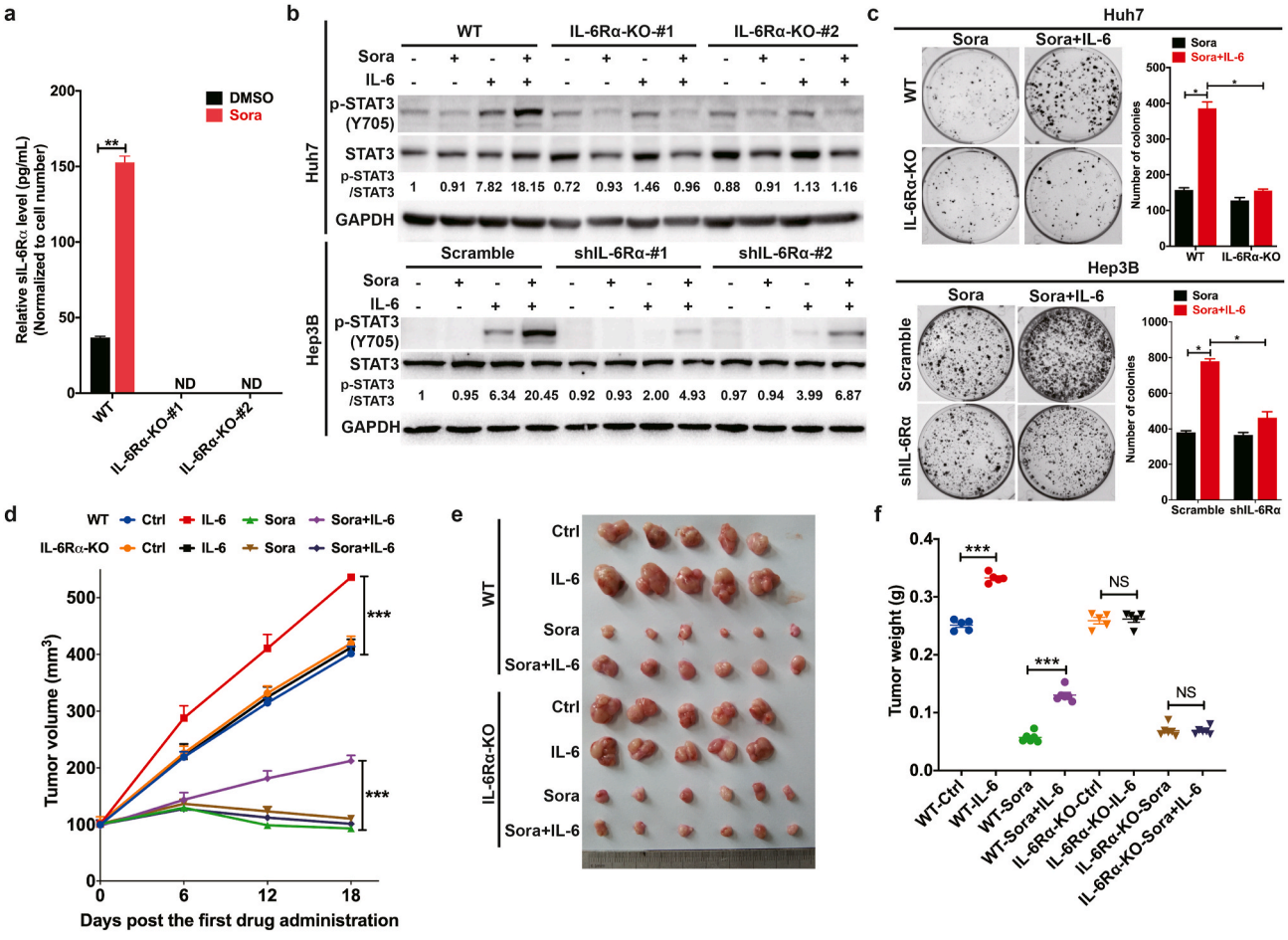

Fig. 2. IL-6Rα is essential for IL-6 mediated sorafenib resistance. (a) Wild-type or IL-6Rα-depleted Huh7 cells were treated with or without 5 μM sorafenib for 36 h. The expression levels of sIL-6Rα were determined by ELISA. (b) Wild-type and IL-6Rα depleted HCC cells were treated as indicated. STAT3 phosphorylation levels at tyrosine-705 and the expression levels of STAT3 were determined by immunoblotting. (c) Colony formation assays were performed in wild-type and IL-6Rα depleted HCC cells treated with 5 μM sorafenib or combination of 5 μM sorafenib and 100 ng/mL IL-6. (d ~ f) 1×10^6 wild-type or IL-6Rα-KO Huh7 cells were inoculated into the right flank of nude mice. When the tumors reached 100 mm^3, the mice were grouped randomly and treated with sorafenib (i.g., 15 mg/kg) with or without IL-6 (i.p., 200 μg/kg) every 3 days. Tumor volumes were monitored every 6 days (d). 18 days later, tumors were harvested (e) and weighed (f). The data are presented as the means ± SEM (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; $n \geq 3$). i.g.:intragastric administration; i.p.: intraperitoneal injection.

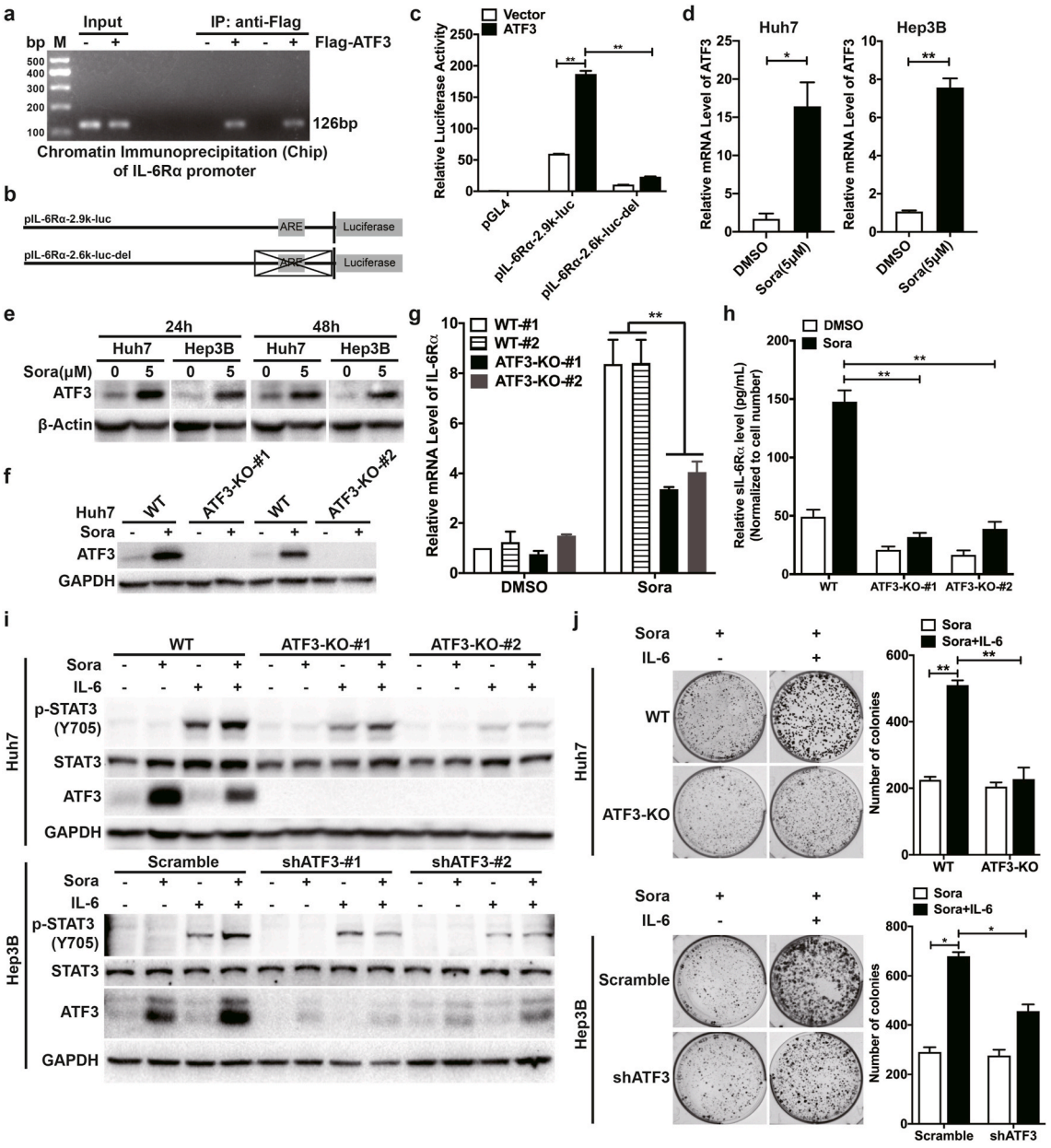

Fig. 3. ATF3 binds to IL-6Rα gene promoter and is essential for IL-6Rα up-regulation by sorafenib in HCC cells. (a) Huh7 cells were infected with a lentivirus expressing Flag-ATF3 and subjected to chromatin immunoprecipitation assay. (b) Schematic representation of the IL-6Rα promoters cloned into the pGL4 vector. (c) ATF3 expression plasmid was co-transfected with pGL4, pIL-6Rα-2.9k-Luc or pIL-6Rα-2.6k-Luc-del into Hela cells. Luciferase assays were performed. (d) Huh7 and Hep3B cells were treated with 5 μM sorafenib for 24 h and subjected to quantitative real-time PCR analysis for ATF3. (e) Hep3B and Huh7 cells were treated with or without 5 μM sorafenib for 24 or 48 h. The expression of ATF3 was determined by immunoblotting. (f) ATF3 was knocked out in Huh7 cells using the CRISPR/Cas9 system. ATF3 protein expression was determined by immunoblotting. (g) Quantitative real-time PCR analysis was performed in wild-type and ATF3-KO Huh7 cells treated with or without sorafenib. The mRNA levels of IL-6Rα were normalized to those of GAPDH. (h) Soluble form of IL-6Rα expression levels were determined by ELISA. (i) Wild-type or ATF3 depleted HCC cells were treated as indicated. STAT3 phosphorylation levels at tyrosine-705, and the expression levels of STAT3 or ATF3 were determined by immunoblotting. (j) Colony formation assays were performed in wild-type or ATF3 depleted HCC cells treated with 5 μM sorafenib or combination of 5 μM sorafenib and 100 ng/mL IL-6. The data are presented as the means ± SEM (*: $P < 0.05$; **: $P < 0.01$; $n = 3$).

presence of sorafenib treatment (Fig. 3i and j). Together, these data suggest that ATF3 binds to the promoter of IL-6Rα and is involved in IL-6Rα induction in response to sorafenib treatment.

3.4. ATF4 is required for ATF3 and IL-6Rα induction by sorafenib

ATF4, a well-known transcription factor of ATF3 and a key regulator of integrated stress response and endoplasmic reticulum stress response, has been shown to be activated by sorafenib [30,31]. Indeed, sorafenib treatment increased ATF4 mRNA expression, as detected by qRT-PCR analysis (Fig. 4a), as well as ATF4 protein levels, as detected by Western blotting analysis (Fig. 4b). Notably, the induction of ATF4 largely depends on inactivation of eIF2α [32], and sorafenib has been shown to inactivate eIF2α through activating PKR-Like endoplasmic reticulum kinase (PERK) [33], an eIF2α kinase. These elegant works suggest that sorafenib might activate ATF4 through PERK-eIF2α pathway. To verify this hypothesis, we investigated whether PERK was required for ATF4 induction by sorafenib. Indeed, either inhibition or genetic ablation of PERK largely suppressed ATF4 induction by sorafenib (Supplementary Fig. S2). These data indicate that sorafenib induces ATF4 through

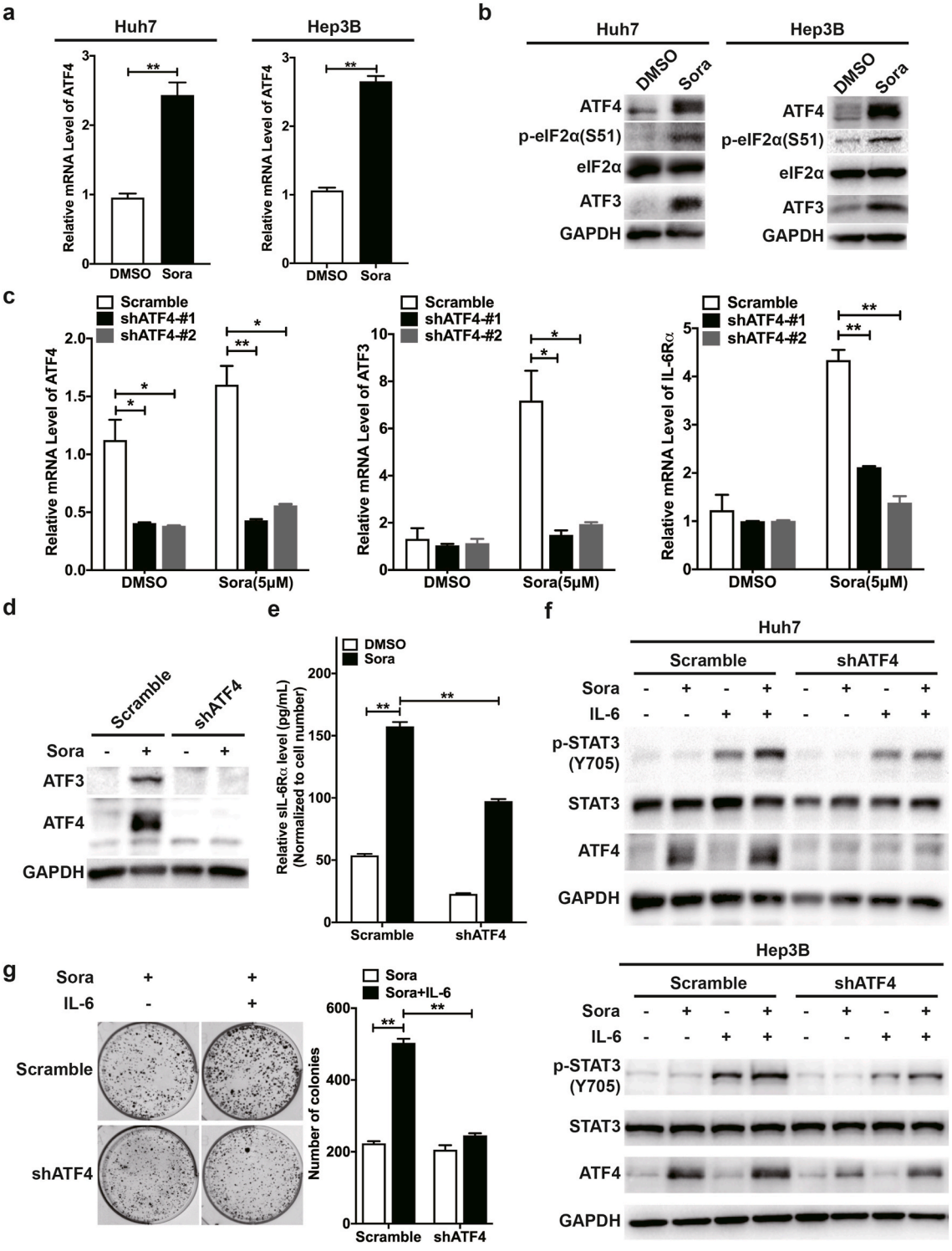

Fig. 4. ATF4 is required for ATF3 and IL-6Rα induction by sorafenib. (a) ATF4 mRNA levels were analyzed by qRT-PCR in Huh7 and Hep3B cells treated with 5 μM sorafenib for 24 h. (b) Phosphorylation of eIF2α at serine-51, and the expression of ATF3, eIF2α and ATF4 were determined by immunoblotting. (c ~ e) Huh7 cells were infected with lentiviruses expressing shRNAs targeting ATF4 and subjected to 5 μM sorafenib treatment for 24 h. ATF4 (left panel), ATF3 (middle panel) and IL-6Rα (right panel) mRNA levels were determined by qRT-PCR (c). ATF4 and ATF3 expression were determined by immunoblotting (d). IL-6Rα expression levels were determined by ELISA assay (e). (f) ATF4 knockdown Huh7, Hep3B and their controlled cells were treated as indicated for 24 h. STAT3 phosphorylation levels at tyrosine-705, and the expression levels of STAT3 and ATF4 were determined by immunoblotting. (g) Colony formation assays were performed in ATF4 depleted Huh7 cells and their parental cells. The mRNA levels of ATF4, ATF3 or IL-6Rα were normalized to GAPDH. The data are presented as the means ± SEM (*: $P < 0.05$; **: $P < 0.01$; $n = 3$).

PERK-eIF2α pathway. Furthermore, ATF4 knockdown largely attenuated the induction of ATF3 and IL-6Rα, both on mRNA and protein expression levels (Fig. 4c–4e). ATF4 depletion also attenuated IL-6 induced STAT3 activation and colony formation under sorafenib treatment (Fig. 4f and g). These data demonstrate that ATF4 activation is required for ATF3 and IL-6Rα induction by sorafenib in HCC cells.

3.5. The ATF4-ATF3-IL-6Rα cascade is also activated in HCC cells following regorafenib treatment

Due to the structural similarity between sorafenib and regorafenib, we investigated whether regorafenib treatment also led to activation of the ATF4-ATF3-IL-6Rα cascade. Indeed, regorafenib up-regulated the mRNA expression of ATF4, ATF3 and IL-6Rα in Huh7 cells (Fig. 5a-5c). The protein levels of either ATF4 or ATF3 were induced in a similar pattern by sorafenib and regorafenib (Fig. 5d). ELISA assays also showed regorafenib up-regulated the protein levels of IL-6Rα (Fig. 5e). Consistently, co-treatment of regorafenib for 24 h also enhanced IL-6 induced STAT3 activation, compared with IL-6 treatment alone (Fig. 5f). ATF4 depletion abolished ATF3 induction (Fig. 5g), while ATF3 or ATF4 depletion attenuated IL-6Rα induction by regorafenib (Fig. 5h and i). Furthermore, depletion of IL-6Rα, ATF3 or ATF4 also attenuated IL-6 induced colony formation capabilities of Huh7 cells in the presence of regorafenib treatment (Fig. 5j). Taken together, these data demonstrate that the ATF4-ATF3-IL-6Rα cascade is also activated by regorafenib.

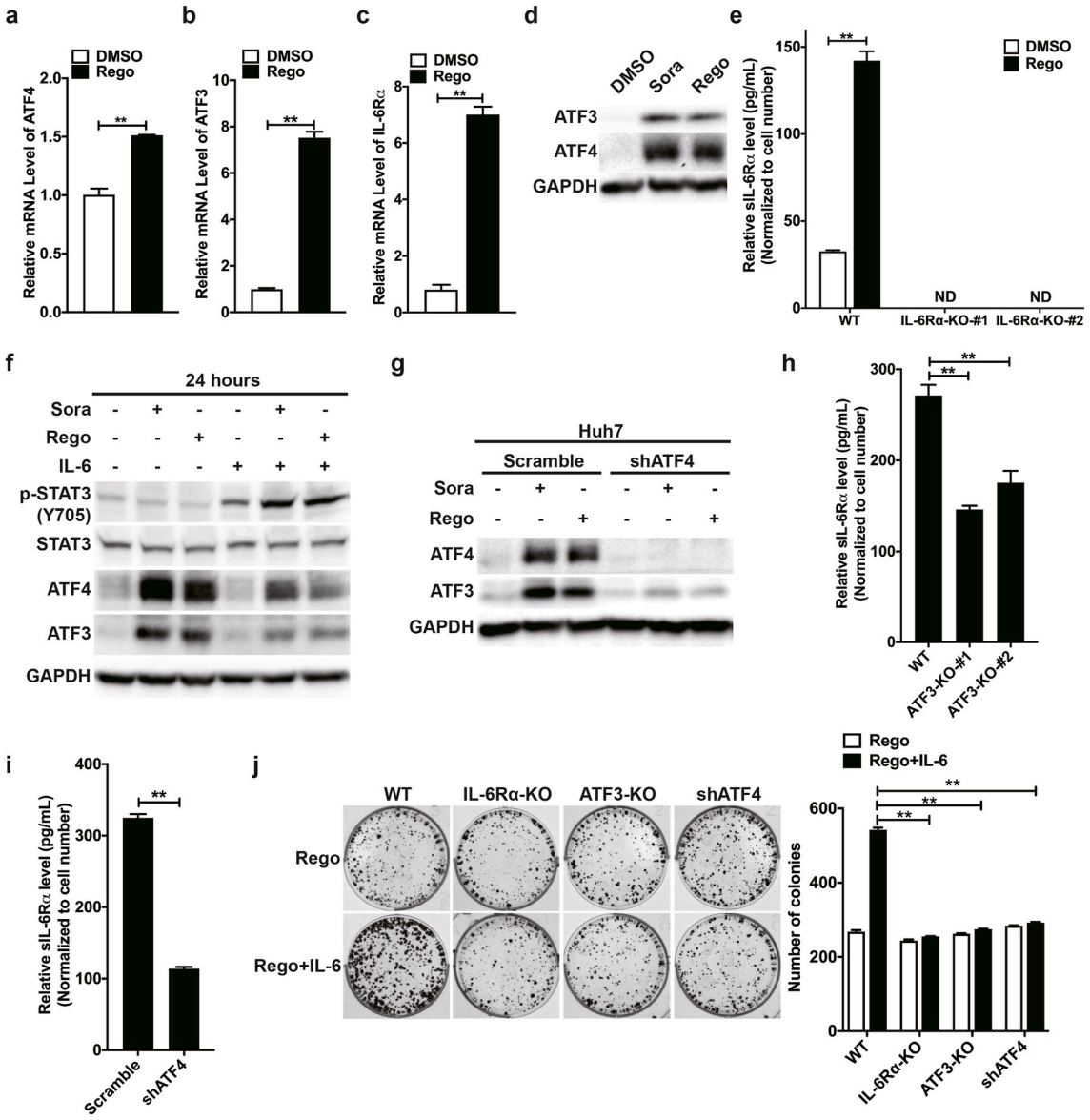

Fig. 5. The ATF4-ATF3-IL-6Rα cascade is also activated by regorafenib. (a, b, c) Huh7 cells were treated with 5 μM regorafenib for 24 h and then subjected to quantitative real-time PCR analysis for ATF4 (a), ATF3 (b) or IL-6Rα (c). The mRNA levels of ATF4, ATF3 and IL-6Rα were normalized to those of GAPDH. (d) ATF3 and ATF4 expression levels were determined by immunoblotting. (e) IL-6Rα expression levels were determined by ELISA assay. (f) Huh7 cells were treated with 5 μM sorafenib, 5 μM regorafenib or 10 ng/mL IL-6 as indicated for 24 h. STAT3 phosphorylation at tyrosine-705, and the expression levels of ATF4, ATF3 and STAT3 were determined by immunoblotting. (g) Scramble and shATF4 Huh7 cells were treated with 5 μM sorafenib or regorafenib for 24 h. ATF4 and ATF3 expressions were determined by immunoblotting. (h, i) Wild-type, ATF3 (h) or ATF4 (i) depleted Huh7 cells were treated with 5 μM regorafenib for 36 h. IL-6Rα expression levels were determined by ELISA. (j) Colony formation assays were performed in wild-type, IL-6Rα, ATF3 or ATF4 depleted Huh7 cells. The data are presented as the means ± SEM (*: $P < 0.05$; **: $P < 0.01$; $n = 3$).

3.6. Blockade of IL-6Rα sensitizes HCC to sorafenib and regorafenib both ex vivo and in vivo

The above results showing that IL-6Rα induction by sorafenib or regorafenib treatment potently enhanced IL-6 mediated drug resistance suggest that IL-6Rα may be a promising anticancer drug target for overcoming sorafenib and regorafenib resistance in HCC. Notably, IL-6Rα monoclonal antibodies against IL-6Rα (Tocilizumab and Sarilumab) have been approved by the FDA to treat human patients with autoimmune diseases, such as rheumatoid arthritis (RA) [34,35]. Thus, we next examined the effect of IL-6Rα antibodies on the efficacy of sorafenib and regorafenib in HCC. We first evaluated the inhibitory effects of

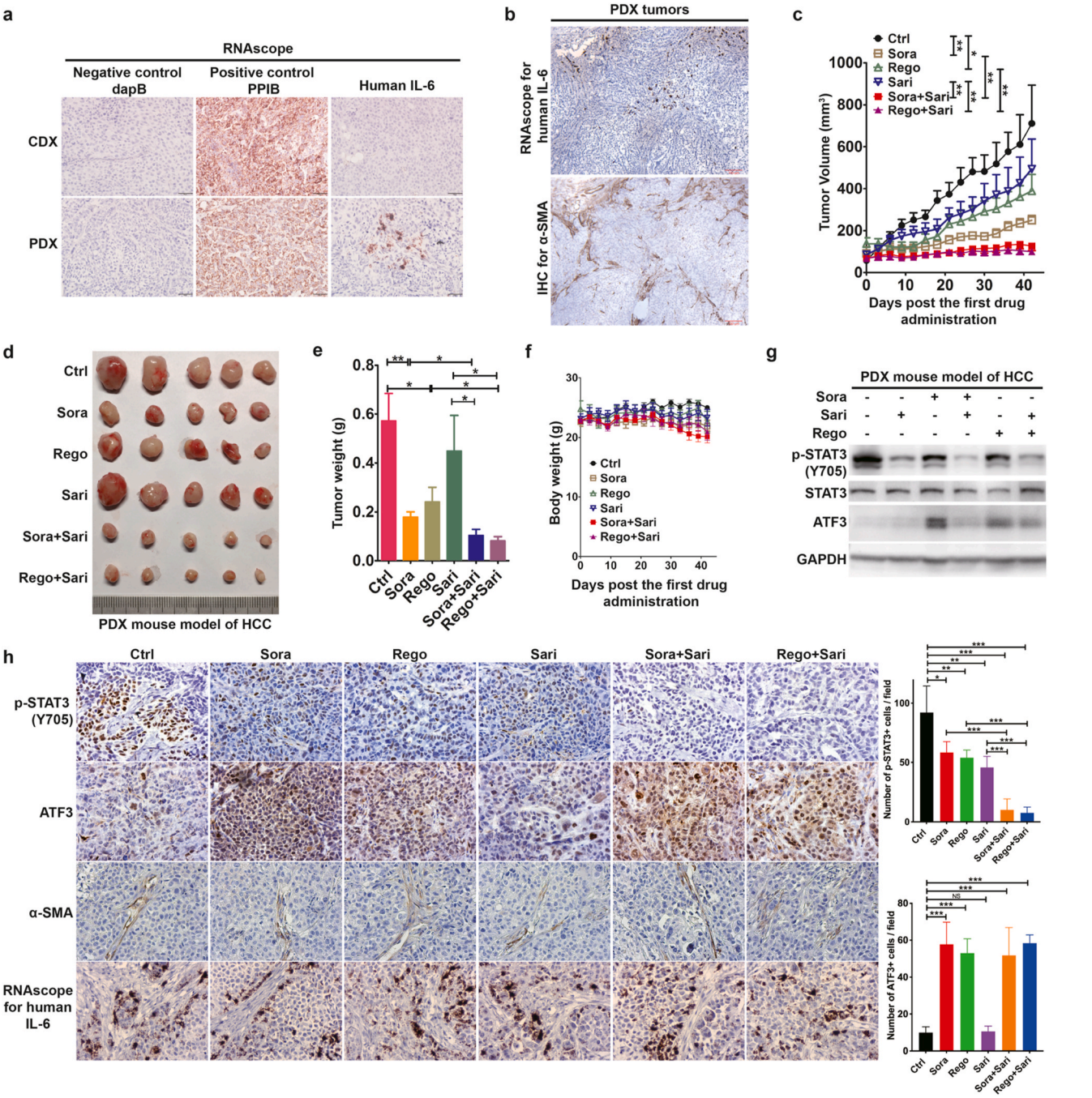

Fig. 6. Blockade of IL-6Rα sensitizes HCC to sorafenib or regorafenib in PDX model. (a) *In situ* hybridization of human IL-6 in CDX and PDX tumors using RNAscope assays (Bars = 50 μm). (b) *In situ* hybridization of human IL-6 mRNA and immunohistochemistry analysis of α-SMA in matched serial sections of PDX tumors (Bars = 100 μm). (c) Patient-derived HCC tumors were inoculated in the flank of nude mice. When the tumors reached ~5 mm in length, the mice were grouped randomly and treated with vehicle, sorafenib (i.g., 15 mg/kg), regorafenib (i.g., 6 mg/kg), Sarilumab (i.p., 5 mg/kg), or their combination every three days. Tumor volumes were monitored every three days. (d ~ e) When the largest tumor reached 1000 mm^3, the mice were sacrificed. The tumors were imaged (d) and weighed (e). (f) The body weights of the mice were also monitored every three days. (g) Phosphorylation of STAT3 at tyrosine-705 and the protein expression levels of ATF3, STAT3 and GAPDH were determined by immunoblotting. (h) Slides from PDX tumors were assessed for the phosphorylation of STAT3 and expression of ATF3 and α-SMA by IHC analysis. Human IL-6 mRNA were detected by RNAscope assay. Of each specimen, five randomly chosen digital snapshots (high-power fields, 400 ×) were taken. Representative pictures are shown. Quantification of p-STAT3(Y705) and ATF3 positive cells was performed using Image-Pro Plus software. The data are presented as the means ± SEM (*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; $n = 5$).

Tocilizumab and Sarilumab on IL-6-induced STAT3 activation in HCC cells. Although both Tocilizumab and Sarilumab blocked the signal transduction from IL-6 to STAT3, the efficacy of Sarilumab was better than that of Tocilizumab, as indicated by STAT3 phosphorylation at tyrosine-705 (Supplementary Figs. S3a and S3b). In line with STAT3 inhibition, Sarilumab largely reversed IL-6 enhanced colony formation in HCC cells under sorafenib or regorafenib treatment (Supplementary Figs. S3c and S3d).

To further explore the effect of Sarilumab on enhancing sorafenib or regorafenib efficacy *in vivo*, we chose a human HCC patient-derived xenograft (PDX) mouse model. The reasons were listed below. Firstly, humanized anti-IL-6Rα antibody does not react to mouse IL-6Rα and mouse IL-6 does not react to human IL-6Rα [36,37]. Therefore, DEN-induced HCC mouse model is not suitable as there is no human IL-6 expressed. Secondly, CDX model lacks an intricate microenvironment, such as human fibroblasts and immune cells which express human IL-6. Thus, CDX is also not a good model to study the efficacy of Sarilumab in treating HCC. Thirdly, PDX has been shown to closely recapitulate clinical responses to treatment [38]. Moreover, RNAscope assay, a novel *in situ* hybridization technology with the probes that specifically recognize human IL-6 but not the mouse one, demonstrated that human IL-6 is expressed in PDX tumors (Fig. 6a). RNAscope and IHC data from matched serial sections indicated that IL-6 could come from, but not limited to, α-SMA (α-smooth muscle actin) positive fibroblasts (Fig. 6b). These data together suggested that PDX model is appropriate for the *in vivo* analysis of IL-6Rα functions and Sarilumab efficacy in HCC. When the PDX tumors reached 100 mm^3, the mice were assigned randomly to six groups to receive treatment with vehicle, sorafenib, regorafenib, Sarilumab, combination of sorafenib and Sarilumab, or combination of regorafenib and Sarilumab. Treatment with sorafenib or regorafenib alone by oral gavage suppressed tumor growth, while intraperitoneal injection of Sarilumab alone also mildly delayed tumor growth. However, the combinational treatments almost completely stopped the growth of the HCC PDX tumors (Fig. 6c). Importantly, the weights of the tumors treated with the combination of Sarilumab and sorafenib or regorafenib were less than half of those treated with sorafenib, regorafenib or Sarilumab alone (Fig. 6d and e). Treatment with sorafenib or regorafenib in the presence or absence of Sarilumab had little effect on the overall body weights of the mice (Fig. 6f). STAT3 phosphorylation at tyrosine-705 was abolished by Sarilumab, while ATF3 was induced by sorafenib or regorafenib, indicated by both immunoblotting and IHC (Fig. 6g and h). By contrast, fibroblast density and human IL-6 expression had no change, indicated by α-SMA staining and RNAscope, respectively (Fig. 6h). These results demonstrate the striking potency of IL-6Rα blockade to synergistically enhance the efficacy of sorafenib and regorafenib in treating HCC.

4. Discussion

According to SHARP and RESORCE data, the overall survival benefits of sorafenib and regorafenib in HCC patients are less than 3 months of additional survival [6,7]. Understanding the underlying mechanism of drug resistance in HCC would help develop novel strategies to improve therapeutic outcome using these drugs. In this study, we found both sorafenib and regorafenib treatment led to induction of IL-6Rα, which enhanced the IL-6 induced STAT3 activation and tumor growth in HCC cells. Based on these findings, we hypothesized that blocking IL-6Rα using anti-IL-6Rα antibodies would sensitize HCC to sorafenib and regorafenib (Fig. 7). Indeed, in our PDX tumor models, Sarilumab greatly enhanced the efficacy of both sorafenib and regorafenib, providing the first preclinical evidence supporting the combination of Sarilumab and sorafenib or regorafenib for patients with advanced HCC to overcome drug resistance.

Anticancer drugs elicit multiple forms of stress, and cancer cells can develop ways to adapt these stresses, which subsequently leads to drug resistance. Sorafenib has been shown to elicit the Nrf2-mediated oxidative stress response and ATF4-mediated ER stress response [33,39]. Nrf2 activation protects HCC cells from sorafenib-induced ferroptosis through activating metallothionein-1G (MT-1G) [40]. Our current study uncovered a novel role of ATF4, the ER stress mediator, in the activation of the IL-6Rα, which enhanced the IL-6-STAT3 pathway to promote cell survival, growth and drug resistance to sorafenib or

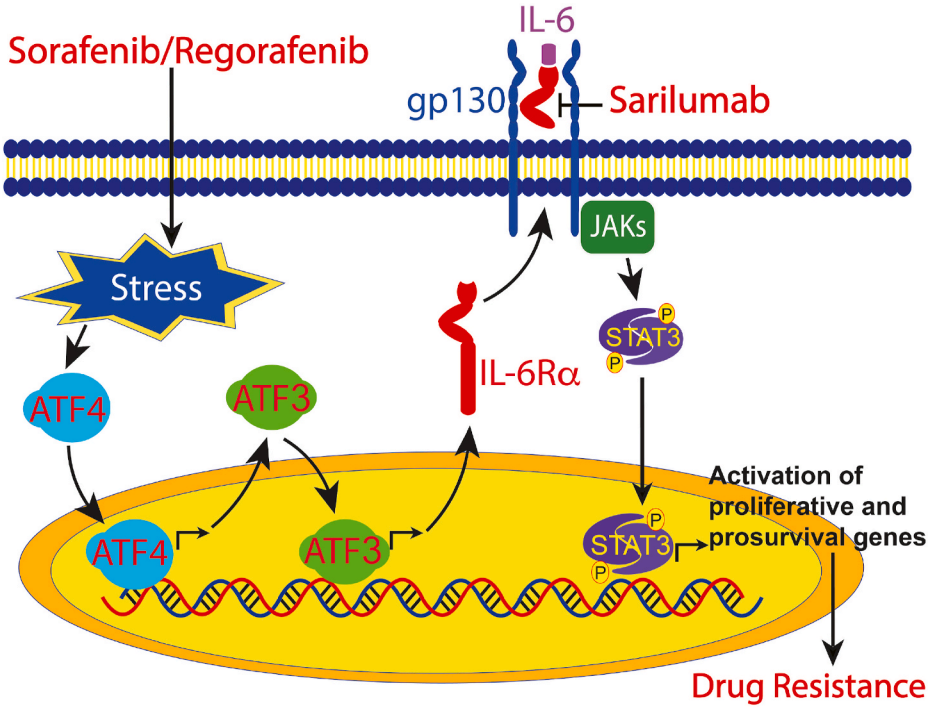

Fig. 7. Graphical Abstract: ATF3-IL-6Rα activation confers sorafenib and regorafenib resistance in HCC, which could be overcome with the IL-6Rα antibody Sarilumab.

regorafenib in HCC. These data suggest a mechanistic link between the ATF4-ATF3-mediated stress response and the IL-6 signaling pathway in HCC drug resistance. Moreover, to our best knowledge, the regulatory mechanism for IL-6Rα expression remains largely unknown. Here, we demonstrate for the first time that IL-6Rα could be transcriptionally activated by xenobiotic stresses through ATF4-ATF3 cascade. ATF3 binds to the promoter of IL-6Rα and activates its transcription. In light of the fact that ATF4-ATF3 pathway could be activated by multiple stimuli, IL-6Rα up-regulation might play a broad spectrum of roles in drug resistance of HCC cells.

Since Rudolf Virchow proposed the hypothesis that inflammation and cancer are linked in the 19th century, and vast amounts of research supported the notion that inflammation is closely linked with cancer development [41]. Recently, accumulating evidence show that inflammation might also play a key role in chemotherapeutic resistance in multiple types of cancer. The effect of IL-6, one of the mediators of inflammation, on sorafenib resistance in HCC is particularly interesting, since IL-6 can antagonize sorafenib in HCC by activating Ras-Erk and Jak/STAT3 signal pathways, which are targeted and suppressed by sorafenib. Moreover, advanced HCC is usually accompanied with cirrhosis and chronic inflammation. IL-6 could be produced by cancer-associated fibroblast (CAF), hepatic stellate cells (HSCs), senescent hepatocytes [42] and macrophages, such as Kupffer cells [43]. While the involvement of IL-6 in sorafenib resistance was suggested before as a novel strategy to overcome the acquisition of sorafenib resistance in HCC [44,45], however mechanistical insights are still lacking. The present study offers further evidence that sorafenib and regorafenib would activate ATF4-ATF3-IL-6Rα pathway, which seems to be a promising targeting axis to improve therapy efficacy in HCC keeping in mind that an FDA-approved IL-6Rα antibody already exists.

Although Epstein-Barr virus-induced gene 3 (EBI3) [46] and CD5 [28] have been implicated to mediate IL-6 signaling independent of IL-6Rα, our data indicate that IL-6Rα is the major receptor for IL-6 in HCC, since IL-6Rα depletion completely abolishes IL-6 signaling. Because sIL-6Rα can facilitate IL-6 signaling in cells that do not express mIL-6Rα, activation of the ATF3-IL-6Rα cascade might not only activate HCC cells themselves, but also adjust microenvironment for cancer cells to survive from sorafenib or regorafenib cytotoxicity. On the other sides, Ciliary Neurotrophic Factor (CNTF) [47,48] and p28(IL-27A) [49–51] were implicated to bind to IL-6Rα. According to our transcriptome sequencing, the expression levels of p28 were weakly up-regulated by sorafenib treatment (Supplementary Fig. S4). Whether sorafenib induced IL-6Rα also enhances sorafenib resistance mediated by this cytokine would be our future work.

In summary, the present study identifies ATF4-ATF3-IL-6Rα as a novel cascade, which is activated in response to sorafenib or regorafenib treatment. Induction of IL-6Rα enhances IL-6 signaling and thereafter promotes sorafenib and regorafenib resistance in HCC, which could be effectively overcome by IL-6Rα antibodies. These findings could also have an immediate and direct impact on HCC patient care given that IL-6Rα antibodies, such as Tocilizumab and Sarilumab, have been clinically used to treat patients with rheumatoid arthritis.

Author contribution statement

Zichan Dai, Xiaohan Wang and Rangxin Peng performed most of the experiments, analyzed the data; Qi Han, Jie Lin and Jichuang Wang developed HCC PDX models; Binghui Zhang, Junjin Lin, Mingting Jiang, Hekun Liu and Tae Ho Lee provided technique support; Kun Ping Lu provided expert advice on experimental design, data interpretation and revising the manuscript; Min Zheng designed the studies, interpreted the data, and wrote the manuscript with inputs from all authors. There are no conflicts of interest.

Funding

This research was supported by the Key Projects of the Natural Science Foundation of Fujian Province (No. 2019J02009), the Joint Science and Technology Innovation Fund Project of Fujian Province (No. 2016Y9043 and 2016Y9045), the Young and Middle-aged Key Personnel Training Program of the Fujian Provincial Health and Family Planning Commission (No. 2017-ZQN-58), the Fujian Environmental Protection Science and Technology Project (No. 2016R015), the Collaborative Innovation Center for Stem Cells Translational Medicine (Fujian 2011 Program), the Joint Funds for the Health and Education of Fujian Province (No. WKJ2016-2-34).

Declaration of competing interest

No potential conflicts of interest were disclosed.

Acknowledgements

We thank Professor Xiao Zhen Zhou for expert advice on experimental design, data interpretation and revising the manuscript. We also thank the Central Core Facility and the Animal Facility of Fujian Medical University for providing equipment and technical support and for supporting our animal studies, respectively.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.canlet.2021.10.024.

References

[1] F. Bray, J. Ferlay, I. Soerjomataram, R.L. Siegel, L.A. Torre, A. Jemal, Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA A Cancer J. Clin. 68 (2018) 394–424.
[2] J.C. Nault, P.R. Galle, J.U. Marquardt, The role of molecular enrichment on future therapies in hepatocellular carcinoma, J. Hepatol. 69 (2018) 237–247.
[3] D. Sia, A. Villanueva, S.L. Friedman, J.M. Llovet, Liver cancer cell of origin, molecular class, and effects on patient prognosis, Gastroenterology 152 (2017) 745–761.
[4] G.M. Keating, Sorafenib: a review in hepatocellular carcinoma, Targeted Oncol. 12 (2017) 243–253.
[5] Regorafenib approved for liver cancer,, Cancer Discov. 7 (2017) 660.
[6] 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, S.I.S. Group, Sorafenib in advanced hepatocellular carcinoma, N. Engl. J. Med. 359 (2008) 378–390.
[7] J. Bruix, S. Qin, P. Merle, A. Granito, Y.H. Huang, G. Bodoky, M. Pracht, O. Yokosuka, O. Rosmorduc, V. Breder, R. Gerolami, G. Masi, P.J. Ross, T. Song, J. P. Bronowicki, I. Ollivier-Hourmand, M. Kudo, A.L. Cheng, J.M. Llovet, R.S. Finn, M.A. LeBerre, A. Baumhauer, G. Meinhardt, G. Han, R. Investigators, Regorafenib for patients with hepatocellular carcinoma who progressed on sorafenib treatment (RESORCE): a randomised, double-blind, placebo-controlled, phase 3 trial, Lancet 389 (2017) 56–66.
[8] S.A. Jones, B.J. Jenkins, Recent insights into targeting the IL-6 cytokine family in inflammatory diseases and cancer, Nature reviews, Immunology 18 (2018) 773–789.
[9] N. Fazel Modares, R. Polz, F. Haghighi, L. Lamertz, K. Behnke, Y. Zhuang, C. Kordes, D. Haussinger, U.R. Sorg, K. Pfeffer, D.M. Floss, J.M. Moll, R.P. Piekorz, M.R. Ahmadian, P.A. Lang, J. Scheller, IL-6 trans-signaling controls liver regeneration after partial hepatectomy, Hepatology 70 (2019) 2075–2091.
[10] L. Giannitrapani, M. Cervello, M. Soresi, M. Notarbartolo, M. La Rosa, L. Virruso, N. D'Alessandro, G. Montalto, Circulating IL-6 and sIL-6R in patients with hepatocellular carcinoma, Ann. N. Y. Acad. Sci. 963 (2002) 46–52.
[11] W. Ohishi, J.B. Cologne, S. Fujiwara, G. Suzuki, T. Hayashi, Y. Niwa, M. Akahoshi, K. Ueda, M. Tsuge, K. Chayama, Serum interleukin-6 associated with hepatocellular carcinoma risk: a nested case-control study, Int. J. Cancer 134 (2014) 154–163.
[12] W.E. Naugler, T. Sakurai, S. Kim, S. Maeda, K. Kim, A.M. Elsharkawy, M. Karin, Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production, Science 317 (2007) 121–124.
[13] T. Ara, R. Nakata, M.A. Sheard, H. Shimada, R. Buettner, S.G. Groshen, L. Ji, H. Yu, R. Jove, R.C. Seeger, Y.A. DeClerck, Critical role of STAT3 in IL-6-mediated drug resistance in human neuroblastoma, Cancer Res. 73 (2013) 3852–3864.

[14] H.Q. Yan, X.B. Huang, S.Z. Ke, Y.N. Jiang, Y.H. Zhang, Y.N. Wang, J. Li, F.G. Gao, Interleukin 6 augments lung cancer chemotherapeutic resistance via ataxia-telangiectasia mutated/NF-kappaB pathway activation, Cancer Sci. 105 (2014) 1220–1227.
[15] I.H. Ham, H.J. Oh, H. Jin, C.A. Bae, S.M. Jeon, K.S. Choi, S.Y. Son, S.U. Han, R. A. Brekken, D. Lee, H. Hur, Targeting interleukin-6 as a strategy to overcome stroma-induced resistance to chemotherapy in gastric cancer, Mol. Cancer 18 (2019) 68.
[16] J. Yang, J. Wang, J. Luo, Decreased IL-6 induces sensitivity of hepatocellular carcinoma cells to sorafenib, Pathol. Res. Pract. 215 (2019) 152565.
[17] Y.Y. Shao, H. Lin, Y.S. Li, Y.H. Lee, H.M. Chen, A.L. Cheng, C.H. Hsu, High plasma interleukin-6 levels associated with poor prognosis of patients with advanced hepatocellular carcinoma, Jpn. J. Clin. Oncol. 47 (2017) 949–953.
[18] K. Yamasaki, T. Taga, Y. Hirata, H. Yawata, Y. Kawanishi, B. Seed, T. Taniguchi, T. Hirano, T. Kishimoto, Cloning and expression of the human interleukin-6 (BSF-2/IFN beta 2) receptor, Science 241 (1988) 825–828.
[19] D. Schmidt-Arras, S. Rose-John, IL-6 pathway in the liver: from physiopathology to therapy, J. Hepatol. 64 (2016) 1403–1415.
[20] M. Garg, M.K. Shanmugam, V. Bhardwaj, A. Goel, R. Gupta, A. Sharma, P. Baligar, A.P. Kumar, B.C. Goh, L. Wang, G. Sethi, The pleiotropic role of transcription factor STAT3 in oncogenesis and its targeting through natural products for cancer prevention and therapy, Med. Res. Rev. 41 (2021) 1291–1336.
[21] J.H. Lee, C.D. Mohan, A. Deivasigamani, Y.Y. Jung, S. Rangappa, S. Basappa, A. Chinnathambi, T.A. Alahmadi, S.A. Alharbi, M. Garg, Z.X. Lin, K.S. Rangappa, G. Sethi, K.M. Hui, K.S. Ahn, Brusatol suppresses STAT3-driven metastasis by downregulating epithelial-mesenchymal transition in hepatocellular carcinoma, J. Adv. Res. 26 (2020) 83–94.
[22] T. Kishimoto, Interleukin-6: from basic science to medicine–40 years in immunology, Annu. Rev. Immunol. 23 (2005) 1–21.
[23] P. Baran, R. Nitz, J. Grotzinger, J. Scheller, C. Garbers, Minimal interleukin 6 (IL-6) receptor stalk composition for IL-6 receptor shedding and IL-6 classic signaling, J. Biol. Chem. 288 (2013) 14756–14768.
[24] L.J. Ho, S.F. Luo, J.H. Lai, Biological effects of interleukin-6: clinical applications in autoimmune diseases and cancers, Biochem. Pharmacol. 97 (2015) 16–26.
[25] M. Peters, G. Blinn, T. Jostock, P. Schirmacher, K.H. Meyer zum Buschenfelde, P. R. Galle, S. Rose-John, Combined interleukin 6 and soluble interleukin 6 receptor accelerates murine liver regeneration, Gastroenterology 119 (2000) 1663–1671.
[26] J. Bergmann, M. Muller, N. Baumann, M. Reichert, C. Heneweer, J. Bolik, K. Lucke, S. Gruber, A. Carambia, S. Boretius, I. Leuschner, T. Becker, B. Rabe, J. Herkel, F. T. Wunderlich, H.W. Mittrucker, S. Rose-John, D. Schmidt-Arras, IL-6 trans-signaling is essential for the development of hepatocellular carcinoma in mice, Hepatology 65 (2017) 89–103.
[27] I.A. Mufazalov, D. Andruszewski, C. Schelmbauer, S. Heink, M. Blanfeld, J. Masri, Y. Tang, R. Schuler, C. Eich, F.T. Wunderlich, S.H. Karbach, J.A. Bluestone, T. Korn, A. Waisman, Cutting edge: IL-6-Driven immune dysregulation is strictly dependent on IL-6R alpha-chain expression, J. Immunol. 204 (2020) 747–751.
[28] C. Zhang, H. Xin, W. Zhang, P.J. Yazaki, Z. Zhang, K. Le, W. Li, H. Lee, L. Kwak, S. Forman, R. Jove, H. Yu, CD5 binds to interleukin-6 and induces a feed-forward loop with the transcription factor STAT3 in B cells to promote cancer, Immunity 44 (2016) 913–923.
[29] R. Dreos, G. Ambrosini, R.C. Perier, P. Bucher, The Eukaryotic Promoter Database: expansion of EPDnew and new promoter analysis tools, Nucleic Acids Res. 43 (2015) D92–D96.
[30] H.Y. Jiang, S.A. Wek, B.C. McGrath, D. Lu, T. Hai, H.P. Harding, X. Wang, D. Ron, D.R. Cavener, R.C. Wek, Activating transcription factor 3 is integral to the eukaryotic initiation factor 2 kinase stress response, Mol. Cell Biol. 24 (2004) 1365–1377.
[31] B. Zhou, Q. Lu, J. Liu, L. Fan, Y. Wang, W. Wei, H. Wang, G. Sun, Melatonin increases the sensitivity of hepatocellular carcinoma to sorafenib through the PERK-ATF4-beclin1 pathway, Int. J. Biol. Sci. 15 (2019) 1905–1920.
[32] K. Pakos-Zebrucka, I. Koryga, K. Mnich, M. Ljujic, A. Samali, A.M. Gorman, The integrated stress response, EMBO Rep. 17 (2016) 1374–1395.
[33] M. Rahmani, E.M. Davis, T.R. Crabtree, J.R. Habibi, T.K. Nguyen, P. Dent, S. Grant, The kinase inhibitor sorafenib induces cell death through a process involving induction of endoplasmic reticulum stress, Mol. Cell Biol. 27 (2007) 5499–5513.
[34] J.S. Smolen, A. Beaulieu, A. Rubbert-Roth, C. Ramos-Remus, J. Rovensky, E. Alecock, T. Woodworth, R. Alten, O. Investigators, Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial, Lancet 371 (2008) 987–997.
[35] T.W. Huizinga, R.M. Fleischmann, M. Jasson, A.R. Radin, J. van Adelsberg, S. Fiore, X. Huang, G.D. Yancopoulos, N. Stahl, M.C. Genovese, Sarilumab, a fully human monoclonal antibody against IL-6Ralpha in patients with rheumatoid arthritis and an inadequate response to methotrexate: efficacy and safety results from the randomised SARIL-RA-MOBILITY Part A trial, Ann. Rheum. Dis. 73 (2014) 1626–1634.
[36] H. Suzuki, K. Yasukawa, T. Saito, M. Narazaki, A. Hasegawa, T. Taga, T. Kishimoto, Serum soluble interleukin-6 receptor in MRL/lpr mice is elevated with age and mediates the interleukin-6 signal, Eur. J. Immunol. 23 (1993) 1078–1082.
[37] S. Shinriki, H. Jono, K. Ota, M. Ueda, M. Kudo, T. Ota, Y. Oike, M. Endo, M. Ibusuki, A. Hiraki, H. Nakayama, Y. Yoshitake, M. Shinohara, Y. Ando, Humanized anti-interleukin-6 receptor antibody suppresses tumor angiogenesis and in vivo growth of human oral squamous cell carcinoma, Clin. Cancer Res.: Off. J. Am. Assoc. Canc. Res. 15 (2009) 5426–5434.
[38] R. Krumbach, J. Schuler, M. Hofmann, T. Giesemann, H.H. Fiebig, T. Beckers, Primary resistance to cetuximab in a panel of patient-derived tumour xenograft models: activation of MET as one mechanism for drug resistance, Eur. J. Cancer 47 (2011) 1231–1243.
[39] X. Sun, Z. Ou, R. Chen, X. Niu, D. Chen, R. Kang, D. Tang, Activation of the p62-Keap1-NRF2 pathway protects against ferroptosis in hepatocellular carcinoma cells, Hepatology 63 (2016) 173–184.
[40] X. Sun, X. Niu, R. Chen, W. He, D. Chen, R. Kang, D. Tang, Metallothionein-1G facilitates sorafenib resistance through inhibition of ferroptosis, Hepatology 64 (2016) 488–500.
[41] K. Taniguchi, M. Karin, NF-kappaB, inflammation, immunity and cancer: coming of age, Nat. Rev. Immunol. 18 (2018) 309–324.
[42] D. Schmidt-Arras, E. Galun, S. Rose-John, The two facets of gp130 signalling in liver tumorigenesis, Semin. Immunopathol. 43 (2021) 609–624.
[43] S. Maeda, H. Kamata, J.L. Luo, H. Leffert, M. Karin, IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis, Cell 121 (2005) 977–990.
[44] Y. Li, G. Chen, Z. Han, H. Cheng, L. Qiao, Y. Li, IL-6/STAT3 signaling contributes to sorafenib resistance in hepatocellular carcinoma through targeting cancer Stem cells, OncoTargets Ther. 13 (2020) 9721–9730.
[45] Y. Jiang, P. Chen, K. Hu, G. Dai, J. Li, D. Zheng, H. Yuan, L. He, P. Xie, M. Tu, S. Peng, C. Qu, W. Lin, R.T. Chung, J. Hong, Inflammatory microenvironment of fibrotic liver promotes hepatocellular carcinoma growth, metastasis and sorafenib resistance through STAT3 activation, J. Cell Mol. Med. 25 (2021) 1568–1582.
[46] S. Chehboun, J. Labrecque-Carbonneau, S. Pasquin, Y. Meliani, B. Meddah, W. Ferlin, M. Sharma, A. Tormo, J.F. Masson, J.F. Gauchat, Epstein-Barr virus-induced gene 3 (EBI3) can mediate IL-6 trans-signaling, J. Biol. Chem. 292 (2017) 6644–6656.
[47] B. Schuster, M. Kovaleva, Y. Sun, P. Regenhard, V. Matthews, J. Grotzinger, S. Rose-John, K.J. Kallen, Signaling of human ciliary neurotrophic factor (CNTF) revisited. The interleukin-6 receptor can serve as an alpha-receptor for CTNF, J. Biol. Chem. 278 (2003) 9528–9535.
[48] E.M. Wagener, M. Aurich, S. Aparicio-Siegmund, D.M. Floss, C. Garbers, K. Breusing, B. Rabe, R. Schwanbeck, J. Grotzinger, S. Rose-John, J. Scheller, The amino acid exchange R28E in ciliary neurotrophic factor (CNTF) abrogates interleukin-6 receptor-dependent but retains CNTF receptor-dependent signaling via glycoprotein 130 (gp130)/leukemia inhibitory factor receptor (LIFR), J. Biol. Chem. 289 (2014) 18442–18450.
[49] S. Crabe, A. Guay-Giroux, A.J. Tormo, D. Duluc, R. Lissilaa, F. Guilhot, U. Mavoungou-Bigouagou, F. Lefouili, I. Cognet, W. Ferlin, G. Elson, P. Jeannin, J. F. Gauchat, The IL-27 p28 subunit binds cytokine-like factor 1 to form a cytokine regulating NK and T cell activities requiring IL-6R for signaling, J. Immunol. 183 (2009) 7692–7702.
[50] C. Garbers, B. Spudy, S. Aparicio-Siegmund, G.H. Waetzig, J. Sommer, C. Holscher, S. Rose-John, J. Grotzinger, I. Lorenzen, J. Scheller, An interleukin-6 receptor-dependent molecular switch mediates signal transduction of the IL-27 cytokine subunit p28 (IL-30) via a gp130 protein receptor homodimer, J. Biol. Chem. 288 (2013) 4346–4354.
[51] X. Yang, H. Hao, Z. Xia, G. Xu, Z. Cao, X. Chen, S. Liu, Y. Zhu, Soluble IL-6 receptor and IL-27 subunit p28 protein complex mediate the antiviral response through the type III IFN pathway, J. Immunol. 197 (2016) 2369–2381.