

Targeting SphK2 reverses acquired resistance of regorafenib in hepatocellular carcinoma

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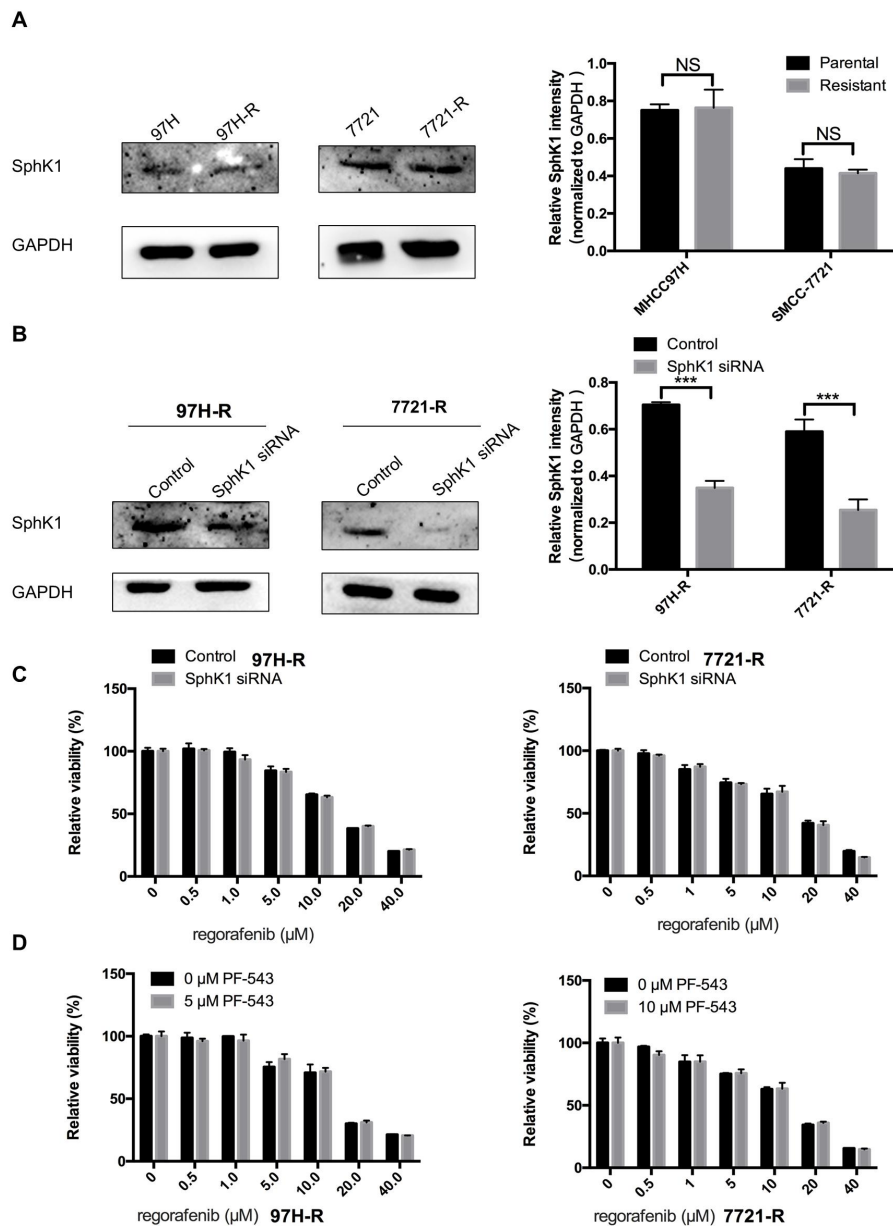
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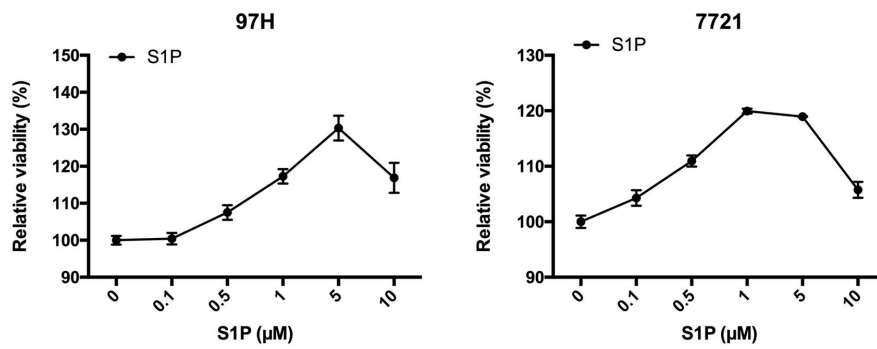
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Supplementary Figure 1



Supplementary Figure 1. The role of SphK1 in regorafenib-resistant HCC cells. (A) SphK1 protein expression levels in parental and regorafenib-resistant HCC cells were determined by western blot analysis. (B) The protein expression levels of SphK1 in regorafenib-resistant HCC cells transfected with SphK1 siRNA or control siRNA were determined by western blot analysis. (C) Regorafenib-resistant HCC cells were transfected with SphK1 siRNA or control siRNA and treated with regorafenib. Cell viability was determined by the CCK-8 assay. (D) Cell viability of regorafenib-resistant HCC cells treated with regorafenib alone or together with PF-543 was determined by the CCK-8 assay. The results were obtained from three independent experiments. The error bars represent mean \pm SD from a representative experiment. *** $p < 0.001$, NS, not significant.

Supplementary Figure 2



Supplementary Figure 2. The effects of S1P on growth of HCC cells. The effect of S1P on HCC cell viability was evaluated by the CCK-8 assay.

Supplementary Table 1. IC₅₀ values of regorafenib in regorafenib-resistant HCC cells transfected with control or SphK1 siRNA.

Cell line	Group	IC ₅₀ (μM)
97H-R	Control	15.11
	SphK1 siRNA	15.08
7721-R	Control	14.31
	SphK1 siRNA	13.76

Supplementary Table 2. IC₅₀ values of regorafenib in regorafenib-resistant HCC cells exposed to vehicle or SphK1 inhibitor PF-543.

Cell line	Group	IC ₅₀ (μM)
97H-R	0 μM PF-543	14.01
	5 μM PF-543	14.70
7721-R	0 μM PF-543	12.61
	10 μM PF-543	12.50

Supplementary Table 3. The suppliers and catalog numbers of all chemical compounds used in the study.

REAGENTS	SUPPLIER	CATALOG NUMBER
Antibodies		
Rabbit monoclonal anti-Bcl2	Cell Signaling Technology (MA, USA)	Cat# 2870P
Rabbit monoclonal anti-cleaved-PARP	Cell Signaling Technology (MA, USA)	Cat# 5625P
Rabbit monoclonal anti-CDK4	Cell Signaling Technology (MA, USA)	Cat# 2906P
Rabbit monoclonal anti-CDK6	Cell Signaling Technology (MA, USA)	Cat# 3136P
Rabbit monoclonal anti-cyclin D1	Cell Signaling Technology (MA, USA)	Cat# 2978S
Rabbit monoclonal anti-STAT3	Cell Signaling Technology (MA, USA)	Cat# 4904S
Mouse monoclonal anti-p-STAT3	Cell Signaling Technology (MA, USA)	Cat# 4113S
Rabbit monoclonal anti-NF-κB p65	Cell Signaling Technology (MA, USA)	Cat# 8242S
Rabbit monoclonal anti-p-NF-κB p65	Cell Signaling Technology (MA, USA)	Cat# 3033S
Rabbit monoclonal anti-SphK1	ProteinTech Group (Wuhan, China)	Cat# 10670-1-AP
Rabbit monoclonal anti-SphK2	ProteinTech Group (Wuhan, China)	Cat# 17096-1-AP
Anti-rabbit IgG, HRP-linked antibody	Cell Signaling Technology (MA, USA)	Cat# 7074
Anti-mouse IgG, HRP-linked antibody	Cell Signaling Technology (MA, USA)	Cat# 7076
Chemicals		
ABC294640	Selleck Chemicals (MA, USA)	Cat# 915385-81-8
Crystal violet	Sigma-Aldrich (MO, USA)	Cat# 548-62-9
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich (MO, USA)	Cat# 67-68-5
Dulbecco's modified Eagle's medium (DMEM)	WISENT (Nanjing, China)	Cat# 319-005-CL
Fetal bovine serum	WISENT (Nanjing, China)	Cat# 087-150
Isoflurane	RWD Life Science (Shenzhen, China)	Cat# 217170701
Lipofectamine™ 2000	Life Technologies (CA, USA)	Cat# 11668019
NP-40 lysis buffer	Beyotime (Shanghai, China)	Cat# P0013F
OptiPro™ SFM	Life Technologies (CA, USA)	Cat# 12309019
Paraformaldehyde	Sigma-Aldrich (MO, USA)	Cat# p6148
Penicillin and streptomycin	WISENT (Nanjing, China)	Cat# 450-201-EI
PMSF Protease Inhibitor	Thermo Fisher Scientific (MA, USA)	Cat# 36978
Polyvinylidene difluoride (PVDF) membranes	Roche Applied Science (Mannheim, Germany)	Cat# 03010040001
Primary Antibody Dilution Buffer	Beyotime (Shanghai, China)	Cat# P0023A
Regorafenib	Selleck Chemicals (MA, USA)	Cat# S1178
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)	Beyotime (Shanghai, China)	Cat# P0690
Sphingosine-1-Phosphate (S1P)	Cayman Chemical (MI, USA)	Cat# 62570
High-Sig ECL Western Blotting Substrate	Biosharp (Beijing, China)	Cat# BL520A
Critical Assays		
Annexin V-FITC/PI double staining assay kit	Vazyme (Nanjing, China)	Cat# A211-02
BCA protein assay Kit	Beyotime (Shanghai, China)	Cat# P0010
Cell Counting Kit-8 assay kit	Selleck Chemicals (MA, USA)	Cat# B34304
Cell cycle assay kit	MultiSciences (Hangzhou, China)	Cat# 70-CCS012
Cell Lines		
BEL-7402 human HCC cells	Cell Bank of the Chinese Academy of	TCHu10

HuH-7 human HCC cells	Sciences (Shanghai, China) Cell Bank of the Chinese Academy of Sciences (Shanghai, China)	TCHu182
MHCC97H human HCC cells	Professor Jia Fan from Zhongshan Hospital of Fudan University	N/A
PLC/PRF/5 human HCC cells	Cell Bank of the Chinese Academy of Sciences (Shanghai, China)	TCHu119
SMMC-7721 human HCC cells	Cell Bank of the Chinese Academy of Sciences (Shanghai, China)	TCHu52
Experimental Models		
Mouse: BALB/c nu/nu	Model Animal Research Center of Nanjing University (Nanjing, China)	N/A
Commercial mice food	Xietong Biological (Nanjing, China)	Cat# 1010010