Combination of Vitamin C and Lenvatinib potentiates antitumor effects in hepatocellular carcinoma cells in vitro

Xinyue Wang [Equal first author, 1], **Songyi Qian** [Equal first author, 1], **Siyi Wang** [1], **Sheng Jia** [1], **Nishang Zheng** [1], **Qing Yao** [Corresp., 1], **Jian Gao** [Corresp. 2]

[1] Zhongshan Hospital (Xiamen), Fudan University, Xiamen, China

[2] Zhongshan Hospital, Fudan University, Shanghai, China

Corresponding Authors: Qing Yao, Jian Gao
Email address: yao.qing@zsxmhospital.com, gao.jian@zs-hospital.sh.cn

Lenvatinib has become a first-line drug in the treatment of advanced hepatocellular carcinoma (HCC). Investigating its use in combination with other agents is of great significance to improve the sensitivity and durable response of Lenvatinib in advanced HCC patients. Vitamin C (L-ascorbic acid, ascorbate, VC) is an important natural antioxidant, which has been reported to show suppressive effects in cancer treatment. Here, we investigated the effect of the combination of VC and Lenvatinib in HCC cells in vitro. We found that treatment of VC alone significantly inhibited the proliferation, migration and invasion in HCC cells. Additionally, VC was strongly synergistic with Lenvatinib in inhibition of the proliferative, migratory and invasive capacities of HCC cells in vitro. In conclusion, our results demonstrate that the combination of VC and Lenvatinib has synergistic antitumor activities against HCC cells, providing a promising therapeutic strategy to improve the prognosis of HCC patients.

1 **Combination of Vitamin C and Lenvatinib Potentiates Antitumor Effects in Hepatocellular**

2 **Carcinoma Cells in Vitro**

3 Xinyue Wang[1,#], Songyi Qian[3, #], Siyi Wang[1], Sheng Jia[1], Nishang Zheng[1], Qing Yao[1,*], Jian

4 Gao[2,*]

5 [1] Department of nutrition, Zhongshan Hospital (Xiamen), Fudan University, Xiamen, Fujian

6 province, China.

7 [2] Department of nutrition, Zhongshan Hospital, Fudan University, Shanghai, China.

8 [3] Department of cardiac surgery, Zhongshan Hospital (Xiamen), Fudan University, Xiamen, Fujian

9 province, China.

10 [#] These authors contributed equally to this work.

11 [*]**Correspondence to:**

12 Qing Yao, Department of nutrition, Zhongshan Hospital (Xiamen), Fudan University, No. 668,

13 JinHu Road, Xiamen, Fujian Province, China, 361015, email: yao.qing@zsxmhospital.com.

14 Jian Gao, Department of nutrition, Zhongshan Hospital, Fudan University, No. 180, Fenglin Road,

15 Shanghai, China, 200032, email: gao.jian@zs-hospital.sh.cn.

16 **Keywords:** vitamin C, Lenvatinib, proliferation, migration, invasion

17 **Running title:** Combination of Lenvatinib and Vitamin C against HCC

19 **Abstract**

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22   the sensitivity and durable response of Lenvatinib in advanced HCC patients. Vitamin C (L-

23   ascorbic acid, ascorbate, VC) is an important natural antioxidant, which has been reported to show

24   suppressive effects in cancer treatment. Here, we investigated the effect of the combination of VC

25   and Lenvatinib in HCC cells in vitro. We found that treatment of VC alone significantly inhibited

26   the proliferation, migration and invasion in HCC cells. Additionally, VC was strongly synergistic

27   with Lenvatinib in inhibition of the proliferative, migratory and invasive capacities of HCC cells

28   in vitro. In conclusion, our results demonstrate that the combination of VC and Lenvatinib has

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30   improve the prognosis of HCC patients.

31

Introduction

32

33   Hepatocellular carcinoma (HCC) is the main histological type of primary liver cancer, which

34   causes about 830,000 deaths every year (Siegel et al. 2020). HCC is mainly caused by chronic

35   infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), alcohol abuse, or non-alcoholic

36   fatty liver disease (NAFLD). Since symptoms of early HCC are usually not obvious, most patients

37   are already in advanced stages at the time of diagnosis and lose the opportunity for local treatment

38   such as radical hepatectomy, radiofrequency ablation, or transarterial therapy (Abbasoglu 2015).

39   Unlike other types of cancers including lung or breast cancer, advanced HCC has few therapeutic

40   options because it is not sensitive to chemotherapy or radiotherapy. Receptor tyrosine kinase

41   signaling pathways, such as the VEGF/VEGFR system, play an important role in regulating

42   angiogenesis. VEGFR plays a central role as a sensor of angiogenesis, which is an important target

43   of several tyrosine kinase inhibitors (TKIs) including Sorafenib and Lenvatinib. However, the

44   efficacy and duration of treatment of these drugs is still limited by acquired or inherent resistance.

45   Therefore, identification of novel enhancers or synergists of TKIs has become an urgent need for

46   clinical treatment of advanced HCC.

47   Lenvatinib has become a first-line drug in the treatment of advanced HCC. Lenvatinib is a multi-

48   target inhibitor for VEGFR1-3, FGFR1-4, PDGFRα/β, C-kit and RET, and exhibits strong

49   antitumor activity (Al-Salama et al. 2019; Hatanaka et al. 2021). The inhibition of FGFR4 by

50   Lenvatinib is considered as a key factor in its antitumor effect (Matsuki et al. 2018). Lenvatinib

51   inhibits both VEGF and FGF pathways and inactivates the proliferative signals in cancer cells,

52   thereby inhibiting tumor growth and angiogenesis (Tohyama et al. 2014; Yamamoto et al. 2014).

53 It can suppress the migration and invasion of cancer cells by regulating the expression of matrix

54 metalloproteinases (MMPs) and tissue inhibitors of MMPs (TIMPs) (He et al. 2018). It also

55 inactivates ERK, AKT and TGF-β pathways, resulting in the suppression of cell migration,

56 epithelial-mesenchymal transformation (EMT) and growth(Zhang et al. 2020). Nevertheless, a

57 total of 85% of patients achieved a partial response or disease stabilization, many patients did not

58 respond or acquired resistance, limiting the overall therapeutic efficacy of Lenvatinib (Kudo et al.

59 2018). For this reason, it is of great significance to improve the sensitivity and durable response

60 of Lenvatinib in advanced HCC patients.

61 Vitamin C (L-ascorbic acid, ascorbate, VC) is an important natural antioxidant with a

62 controversial history in cancer treatment. In the 1970s, Pauling and Cameron conducted clinical

63 trials showing that intravenous injection of VC effectively prolong survival in patients with

64 advanced cancer (Cameron & Campbell 1974; Cameron & Pauling 1976). However, the Mayo

65 study found that oral VC did not show any benefit for tumors (Creagan et al. 1979; Moertel et al.

66 1985). It was later realized that the route of VC administration was the key reason for this

67 difference, with intravenous injection of VC producing much higher plasma concentrations than

68 oral VC (Ngo et al. 2019). A recent study reported that VC is able to kill cancer stem cells, and

69 intravenous injection of VC has a significant effect on prolonging tumor-free survival of patients

70 with HCC (Lv et al. 2018). However, whether VC enhances the anticancer effect of Lenvatinib

71 remains unclear. In the present study, we aimed to evaluate the efficacy of combination of

72 Lenvatinib and VC against HCC. We found that the combination of Lenvatinib and VC exhibits

73 significant suppressive activity in HCC cells in vitro. Our study indicates that the combinational

74 therapy of Lenvatinib and VC may be a promising strategy for HCC patients.

75 **Materials and methods**

76 **Cell culture and drugs**

77   Hep3B, Huh7 and SK-Hep-1 cells were obtained from Fujian Provincial Key Laboratory of

78 Chronic Liver Disease and Hepatocellular Carcinoma, Xiamen University. All cells were cultured

79 in DMEM medium (Gibco) supplemented with 10% fetal bovine serum (FBS, Vivacell) at 37℃

80 in an atmosphere containing 5% CO_2. The authentication of these cell lines used in this present

81 study was performed via comparisons with the STR database. VC and lenvatinib were purchased

82 from Selleck Chemicals and MedChemExpress Company, respectively.

83 **Ethical statement**

84 This study was approved by the Ethics Committee of Xiamen Branch, Zhongshan Hospital, Fudan

85 University (ethics approval number: no. 2021–0910-DN).

86 **Colony formation assay**

87   2000 cells were seeded into 6-well plates, treated with drug or drug vehicle and incubated for

88 about 7-14 days until colonies were visible. Cell culture medium with different concentration of

89 VC and/or lenvatinib were changed every three days during this period. Then, cells were fixed

90 with 4% paraformaldehyde for 15 min and stained with 0.1% crystal violet solution for 30 min.

91 The number of visible colonies (＞50 cells/colony) were counted.

92 **Cell viability detection**

93   Cell viability was assessed using cell counting kit-8 (CCK-8). 2000 cells were seeded into each

94 well of 96-well plates in 100 μl culture medium and incubated overnight. On the following day,

95   cells were treated with drug or drug vehicle. At indicated time point, 10 μl CCK-8 reagent was

96   added to each well and incubated for another 1 hours. The absorbance was measured at 450 nm

97   with a multifunctional microplate reader (Thermo).

98   **Transwell assay**

99   Cells were pre-treated with drug or drug vehicle for 48 h. To detect cell migration, 1×10^5cells

100   were resuspended in serum-free DMEM and added into upper chambers in a transwell insert

101   (Corning). To detect cell invasion, 2×10^5cells were resuspended in serum-free DMEM and added

102   into upper chambers in a transwell insert coated with Matrigel (BD Biosciences). 500 μl DMEM

103   medium supplemented with 10% FBS was added to the lower chamber. After incubation for 24 h,

104   cells on the top of the membrane were wiped off with a cotton swab. Cells that migrated through

105   the membrane were fixed with 4% paraformaldehyde for 15 min, stained with 0.1% crystal violet

106   solution for 30 min and counted under a microscope.

107   **qRT-PCR assay**

108   Total RNA from cells was extracted by using EasyPure® RNA Kit (Transgene, Beijing, China).

109   1 μg RNA was reversely transcribed using TransScript® Uni All-in-One First-Strand cDNA

110   Synthesis SuperMix for qPCR (One-Step gDNA Removal) (Transgene, Beijing, China). Then,

111   qRT-PCR was performed in the Lightcycle Real-Time PCR System (Roche) using FastStart

112   Universal SYBR Green Master (Rox) (Roche). The gene-specific primers are shown as follow: c-

113   MET-forward: CCACGGGACAACACAATACA,   c-MET-reverse:

114   TAAAGTGCCACCAGCCATAG;   ACTB-forward: GGAAATCGTGCGTGACATTAAG,

115   ACTB-reverse: AGCTCGTAGCTCTTCTCCA. ACTB mRNA was taken as internal reference.

116 **Statistics**

117 Data are presented as mean ± standard deviation (SD). All comparisons of means were calculated

118 by using ANOVA tests with Tukey HSD correction for multiple means comparisons. A P value

119 less than 0.05 was considered statistically significant.

120 **Results**

121 **VC inhibits the proliferation, migration and invasion of HCC cells**

122 First, we treated three different HCC cell lines with various concentration of VC, and the

123 concentration-survival curves were plotted. The results showed that the 50% inhibitory

124 concentration (IC_{50}) of VC in Hep3B and SK-Hep-1 cells was much lower than that in Huh7 cells

125 (Figure 1A), suggesting that Hep3B and SK-Hep-1 cells were more sensitive to VC than Huh7

126 cells. Treatment with VC suppressed HCC cell proliferation in a does- and time-dependent manner

127 (Figure 1B). Similarly, the VC treatment repressed the colony formation ability of HCC cells

128 (Figure 1C). However, VC did not show an obvious cytotoxic effect on THLE-2 cells, a normal

129 liver cell line (Supplemental Figure 1).

130 The effect of VC on the migration and invasion was also measured. The results of transwell

131 assays demonstrated that the migratory and invasive abilities of HCC cells was inhibited by the

132 treatment of VC in a does-dependent manner (Figure 2A and 2B).

133 **VC enhances the anti-proliferative activity of Lenvatinib in HCC cells**

134 Next, we determined whether VC enhanced the effect of Lenvatinib on cell survival. we treated

135 HCC cells with various concentrations of VC, Lenvatinib or a combination of both for 48 h.

136 Treatment with Lenvatinib alone significantly inhibited cell survival, while cotreatment of VC and

137 Lenvatinib had a greater inhibitory effect (Figure 3A). Upon co-administration with VC, the IC_{50}

138 values of Lenvatinib were significantly decreased. Furthermore, cell proliferation was examined

139 using CCK-8 and colony formation assays, and the results showed that the combination of VC and

140 Lenvatinib exerts a more significant anti-proliferative effect on HCC cells (Figure 3B-3C).

141   We then evaluated their synergism using the Chou-Talalay method with the Calcusyn software

142 (Biosoft, Cambridge, UK). As shown in Table 1, a combination index (CI) less than 1 indicates

143 the drug combination has synergism. The CI value can reach as low as 0.6883, demonstrating a

144 moderate synergistic activity for this drug combination.

145 **VC reinforces the anti-metastatic effect of Lenvatinib in HCC cells**

146   Although previous studies have reported that Lenvatinib can attenuated the tumor metastasis,

147 whether its combination with VC enhanced this effect remains unknown. The transwell assays

148 demonstrated that the cellular migration and invasion were significantly inhibited after treatment

149 with the combination of VC and Lenvatinib compared with treatment with either of these drugs

150 alone (Figure 4A and 4B).

151 **VC suppresses the c-Met mRNA expression**

152   Finally, we preliminarily investigated the underlying molecular mechanism. Previous study

153 reported that VC represses the transcription of c-MET (Chan et al. 1999; Lorenzato et al. 2020;

154 Pathi et al. 2011; Ye et al. 2019). C-MET has been found to be important for the resistance of

155 Lenvatinib (Fu et al. 2020b; Nakagawa et al. 2014). Therefore, we detected the effect of VC

156 treatment on c-MET mRNA expression. The results of qRT-PCR showed that administration of

157 VC significantly downregulated the mRNA expression of c-MET (Figure 5).

Discussion

158

159   The inhibition of one canonical signaling will activate another compensatory pathway in the

160   treatment of cancer, leading to the resistance against drugs. Therefore, combination therapies

161   applying agents aiming at different targets is prevalent in cancer managements. In 2018,

162   Lenvatinib was approved by the FDA as a first-line treatment for advanced patients with HCC.

163   However, some patients with advanced HCC who received Lenvatinib therapy acquired drug

164   resistance after a period of time. Recently, the underlying mechanism of Lenvatinib therapy in

165   HCC has been revealed. In specific, inhibition of fibroblast growth factor receptor (FGFR) by

166   Lenvatinib treatment leads to feedback activation of the EGFR-PAK2-ERK5 signaling axis. The

167   combination of the EGFR inhibitor Gefitinib or Erlotinib and Lenvatinib displays enhanced anti-

168   proliferative effects (He et al. 2022; Jin et al. 2021). HGF/c-MET signaling is closely associated

169   with drug resistance in cancer. In HCC cells with high c-MET expression, HGF reduces the

170   antiproliferative and anti-invasive effects of Lenvatinib. Using the c-MET inhibitor PHA-665752

171   or Golvatinib is capable to abolish HGF-induced Lenvatinib resistance (Fu et al. 2020b; Nakagawa

172   et al. 2014). Moreover, using CRISPR/Cas9 library screening method, two key resistance genes,

173   neurofibromin 1(NF1) and dual specificity phosphatase 9 (DUSP9), are screened out as critical

174   drivers for Lenvatinib resistance in HCC cells. Trametinib, an inhibitor of MEK pathway, can be

175   used to reverse Lenvatinib resistance mediated by NF1 and DUSP9 loss in HCC cells (Lu et al.

176   2021). These findings suggest that targeting the resistance-associated molecules or pathway will

177   improve the anti-tumor effect of Lenvatinib therapy for HCC.

178   VC is transported into cells primarily through sodium-dependent vitamin C-specific transporters

179 (SVCTs). For liver, SVCT-2 is a key protein responsible for VC uptake, and the inhibitory effect

180 of VC on HCC cells is related to the expression of SVCT-2 (Lv et al. 2018). Here, we found that

181 Hep3B and SK-Hep-1 cells were more sensitive to VC treatment than Huh7 cells, which may due

182 to the differential expression levels of SVCT-2. Magnesium supplementation can activate SVCT-

183 2 and increase the Vmax value of SVCT-2, which show synergistic anticancer effects with VC in

184 cancer cells with low expression level of SVCT-2 (Cho et al. 2020). Thus, cotreatment with $MgSO_4$

185 or $MgCl_2$ solution may improve the sensitivity of VC in Huh7 cells.

186   VC increases ROS levels in tumor cells, causing DNA damage and ATP depletion, leading to

187 cell cycle arrest and apoptosis (Lv et al. 2018). In addition, VC plays an important role in the

188 composition, structure, and biomechanical characteristics of the extracellular matrix by regulating

189 the amount and molecular structure of collagen, and suppresses hypoxia-inducible factor (HIF),

190 thereby inhibiting tumor metastasis and angiogenesis (Fu et al. 2020a). Consistently, we found that

191 VC significantly inhibited the proliferation, migration and invasion of HCC cells. Notably, VC

192 inactivates PAK2, represses the transcription of c-Met and inhibits the EGFR-MAPK/ERK

193 signaling(Chan et al. 1999; Lorenzato et al. 2020; Pashirzad et al. 2021; Pathi et al. 2011; Ye et al.

194 2019). These important molecules and signaling pathways are already demonstrated to be closely

195 associated with Lenvatinib resistance and metastasis in cancer cells(Fu et al. 2020b; Jin et al. 2021;

196 Nakagawa et al. 2014; Shao et al. 2020). Our results further confirmed the suppressive effect of

197 VC on c-MET mRNA expression in HCC cells. Altogether, these findings suggested that VC may

198 improve the resistance of Lenvatinib therapy and repress metastasis in HCC via regulating c-MET.

199 On the other hand, our results of this study showed that treatment with the combination of VC and

200 Lenvatinib showed a better anti-proliferative and anti-metastatic effect compared with treatment

201 with either of these drugs alone. However, the *in vivo* experiments and exact mechanism need

202 further investigation.

203   Recent studies have found that Lenvatinib combined with anti-PD-1 therapy plays a unique

204 immunomodulatory role by activating immune pathways, reducing Treg cell infiltration and

205 inhibiting TGFβ signaling (Torrens et al. 2021). In addition, Lenvatinib reduces PD-L1 level of

206 HCC cells and Treg differentiation by blocking FGFR4, which improves anti-PD-1 efficacy.

207 Lenvatinib enhances the proteasome degradation process of PD-L1 in HCC cells by blocking

208 FGFR4-GSK3β pathway. The sensitivity of IFN-γ-pretreated HCC cells to the killing effect of T

209 cell can be restored by targeting FGFR4. On the other hand, interleukin-2 (IL-2) levels increased

210 after anti-PD-1 treatment, but Lenvatinib blocked IL-2-mediated Treg differentiation by targeting

211 FGFR4 and inhibiting signal transduction and STAT5 phosphorylation (Yi et al. 2021).

212 Interestingly, VC also enhances the effect of anti-PD-1 immunotherapy, which may be achieved

213 by inhibiting IL-6-mediated pD-L1 protein stability (Chan et al. 2019; Luchtel et al. 2020). These

214 findings suggest that combination of VC and Lenvatinib may augment antitumor immune response

215 of anti-PD-1 in HCC.

216   In sum, our current study implicated that the cotreatment of VC and Lenvatinib can improve

217 treatment efficacy. Nonetheless, further *in vivo* study, as well as clinical evidences are still needed

218 to confirm this clinical translation.

219 **Conflict of interest**

220   None.

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Authors' contributions

228 Xinyue Wang, Songyi Qian, Siyi Wang, Sheng Jia and Nishang Zheng performed all the

229 experiments. Xinyue Wang wrote the manuscript. Jian Gao and Qing Yao contributed to data

230 analysis.

Availability of data and materials

232 The datasets used during this research are available.

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336

337   **Figure legend**

338   **Figure 1. VC inhibits the proliferation, migration and invasion of HCC cells.**

339   A. Hep3B, SK-Hep-1 and Huh7 cells were treated with various concentrations of VC for 48h.

340   IC_{50} value of VC in HCC cell lines was then determined.

341   B. Hep3B, SK-Hep-1 and Huh7 cells were treated with various concentrations of VC. At different

342   time points, the cell proliferation was determined by CCK-8 assay.

343   C. The clone formation of Hep3B, SK-Hep-1 and Huh7 cells treated with various concentrations

344   of VC were detected. The representative images and statistical results were shown.

345    $*p<0.05$, $**p<0.01$, $***p<0.001$.

346

347   **Figure 2. VC inhibits the proliferation, migration and invasion of HCC cells.**

348   Hep3B, SK-Hep-1 and Huh7 cells were treated with various concentrations of VC for 48 hr. A

349   transwell assay was then conducted to examine the migratory and invasive capabilities of the HCC

350   cells. The representative images and statistical results of the transwell assays were shown.

351    $**p<0.01$, $***p<0.001$.

352

353   **Figure 3. VC enhances the anti-proliferative activity of Lenvatinib in HCC cells**

354   A. Hep3B, SK-Hep-1 and Huh7 cells were treated with various concentrations of VC, Lenvatinib,

355   or both of the drugs for 48h. IC_{50} value of VC in HCC cell lines was then calculated.

356   B. Hep3B, SK-Hep-1 and Huh7 cells were treated with VC and Lenvatinib individually or in

357   combination. At different time points, the cell proliferation was determined by CCK-8 assay.

358   (Hep3B: 0.5mM VC, 4μM LEN; SK-Hep-1: 1.25mM VC, 8μM LEN; Huh7: 2mM VC, 8μM

359   LEN)

360   C. The clone formation of Hep3B, SK-Hep-1 and Huh7 cells treated with VC and Lenvatinib

361   individually or in combination were detected. The representative images and statistical results

362   were shown. (Hep3B: 0.5mM VC, 4μM LEN; SK-Hep-1: 1.25mM VC, 8μM LEN; Huh7:

363   1mM VC, 8μM LEN)

364   *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

365

366   **Figure 4. VC reinforces the anti-metastatic effect of Lenvatinib in HCC cells.**

367   Hep3B, SK-Hep-1 and Huh7 cells were treated with VC and Lenvatinib individually or in

368   combination for 48 hr. A transwell assay was then conducted to examine the migration (A) and

369   invasion (B) of the HCC cells. The representative images and statistical results of the transwell

370   assays were shown. (Hep3B: 0.5mM VC, 4μM LEN; SK-Hep-1: 0.5mM VC, 8μM LEN; Huh7:

371   1mM VC, 8μM LEN) *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

372

373   **Figure 5. VC suppresses the c-Met mRNA expression**

374   Hep3B, SK-Hep-1 and Huh7 cells were treated with various concentrations of VC. 48 hours later,

375   the c-MET mRNA levels were measured using qRT-PCR assay. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure 1

Figure 1. VC inhibits the proliferation, migration and invasion of HCC cells.

A. Hep3B, SK-Hep-1 and Huh7 cells were treated with various concentrations of VC for 48h. IC50 value of VC in HCC cell lines was then determined.

B. Hep3B, SK-Hep-1 and Huh7 cells were treated with various concentrations of VC. At different time points, the cell proliferation was determined by CCK-8 assay.

C. The clone formation of Hep3B, SK-Hep-1 and Huh7 cells treated with various concentrations of VC were detected. The representative images and statistical results were shown.

*p<0.05, **p<0.01, ***p<0.001.
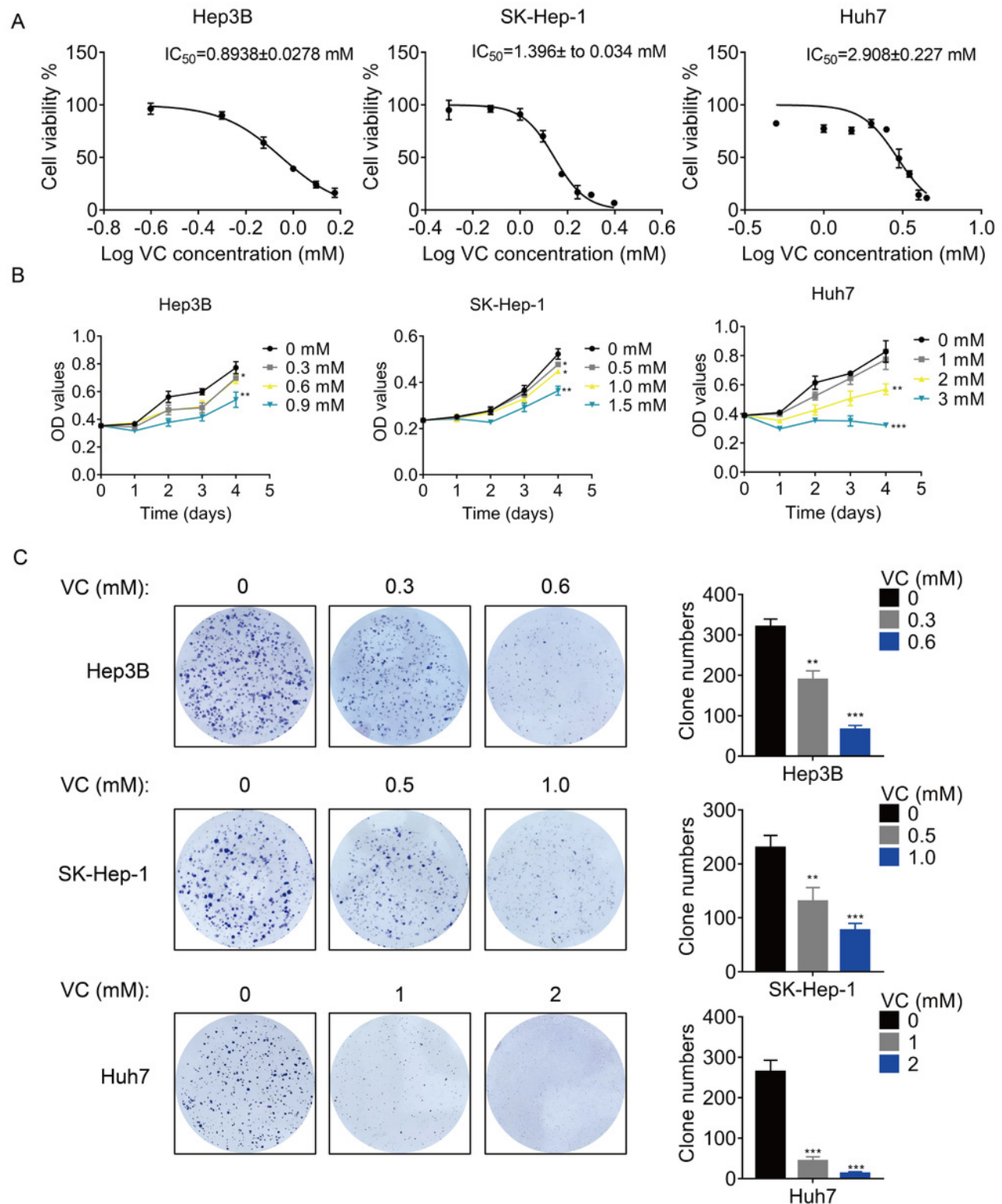
Figure 2

Figure 2. VC inhibits the proliferation, migration and invasion of HCC cells.

Hep3B, SK-Hep-1 and Huh7 cells were treated with various concentrations of VC for 48 hr. A transwell assay was then conducted to examine the migratory and invasive capabilities of the HCC cells. The representative images and statistical results of the transwell assays were shown. **p<0.01, ***p<0.001.
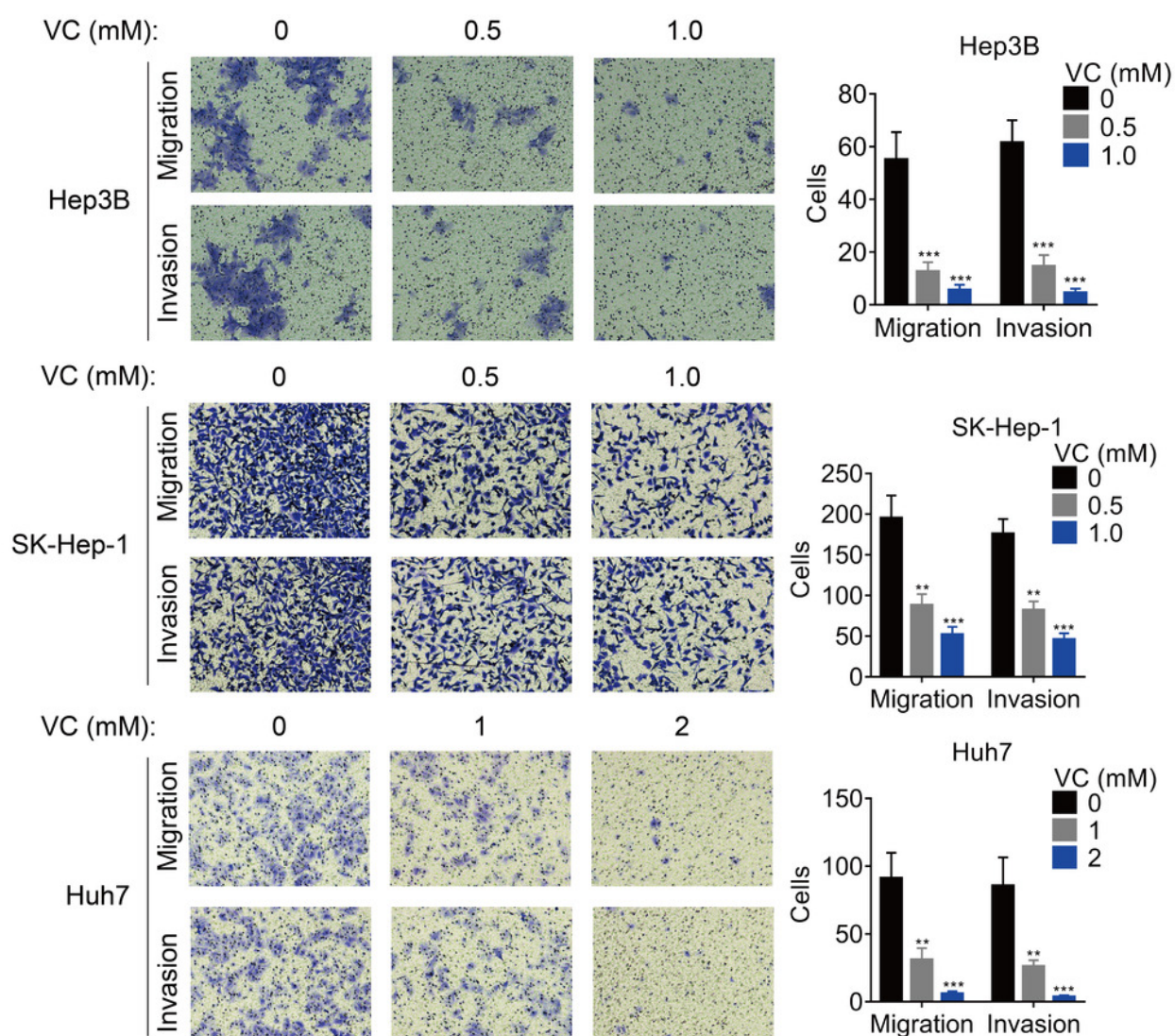
Figure 3

Figure 3. VC enhances the anti-proliferative activity of Lenvatinib in HCC cells

A. Hep3B, SK-Hep-1 and Huh7 cells were treated with various concentrations of VC, Lenvatinib, or both of the drugs for 48h. IC50 value of VC in HCC cell lines was then calculated.

B. Hep3B, SK-Hep-1 and Huh7 cells were treated with VC and Lenvatinib individually or in combination. At different time points, the cell proliferation was determined by CCK-8 assay.

C. The clone formation of Hep3B, SK-Hep-1 and Huh7 cells treated with VC and Lenvatinib individually or in combination were detected. The representative images and statistical results were shown.

*p<0.05, **p<0.01, ***p<0.001.

A

VC (mM)	IC$_{50}$ of LEN (μM)
0	9.72±1.87
0.25	9.07±1.77
0.5	2.47±0.43
0.75	0.65±0.21

VC (mM)	IC$_{50}$ of LEN (μM)
0	22.96±2.38
1	19.06±3.15
1.25	8.87±1.88
1.5	3.10±0.68

VC (mM)	IC$_{50}$ of LEN (μM)
0	24.97±4.25
1	22.04±3.82
2	15.71±4.66
3	7.57±2.64

B

C
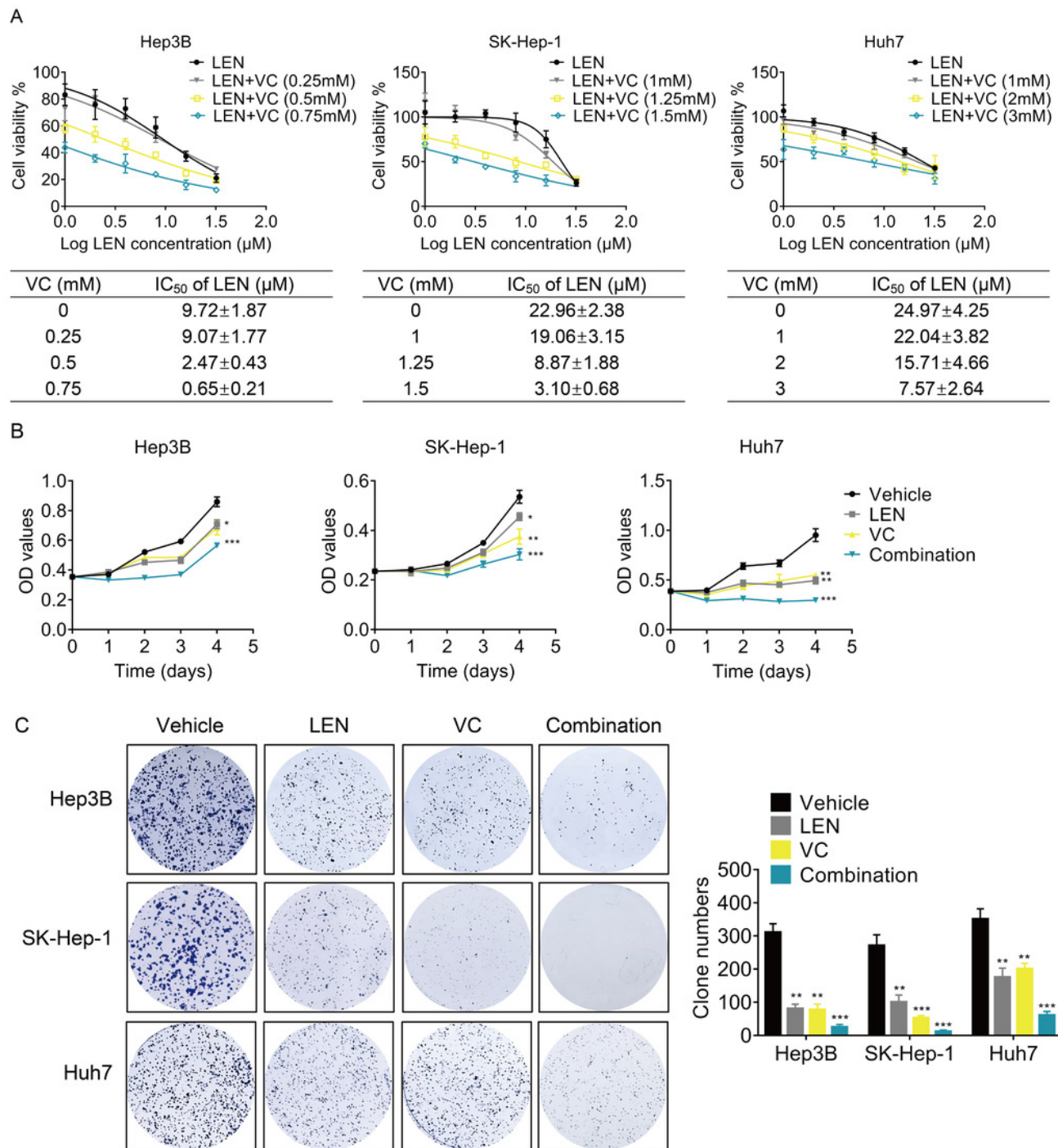
Figure 4

Figure 4. VC reinforces the anti-metastatic effect of Lenvatinib in HCC cells.

Hep3B, SK-Hep-1 and Huh7 cells were treated with VC and Lenvatinib individually or in combination for 48 hr. A transwell assay was then conducted to examine the migration (A) and invasion (B) of the HCC cells. The representative images and statistical results of the transwell assays were shown. *p<0.05, **p<0.01, ***p<0.001.
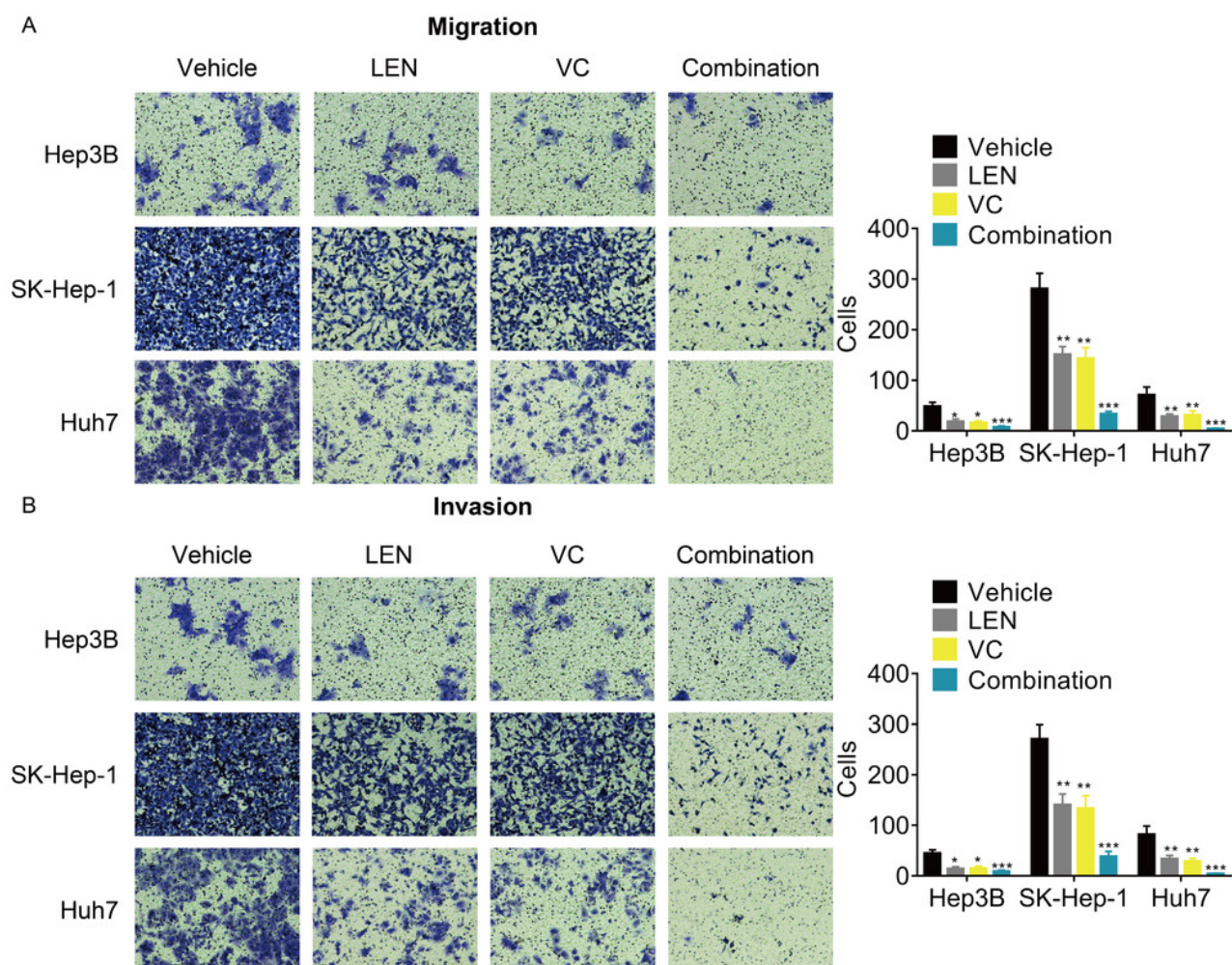
Figure 5

Figure 5. VC suppresses the c-Met mRNA expression

Hep3B, SK-Hep-1 and Huh7 cells were treated with various concentrations of VC. 48 hours later, the c-MET mRNA levels were measured using qRT-PCR assay. *p<0.05, **p<0.01, ***p<0.001.
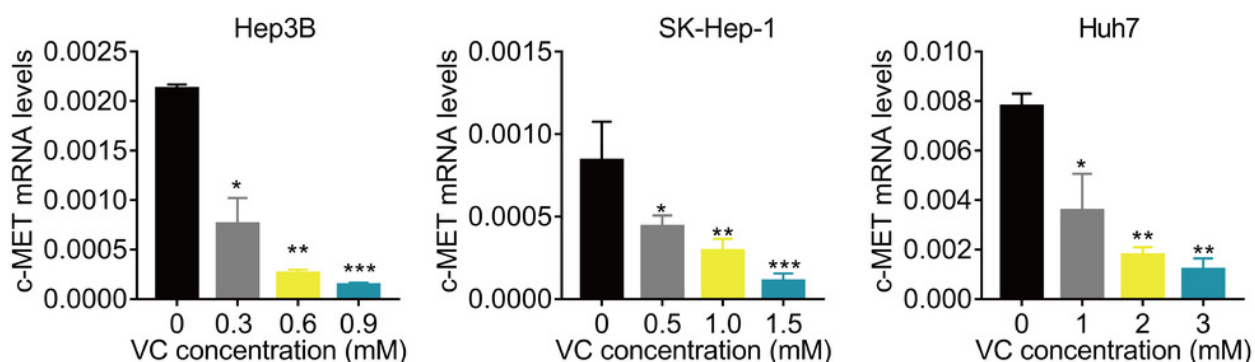
Table 1. The combination index calculated by CalcuSyn software

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	Lenvatinib (μM)	Vitamin C (mM)	CI
	4	0.25	1.4424
Hep3B	8	0.5	0.8928
	16	0.75	0.7166
	4	1	1.3114
SK-Hep-1	8	1.25	0.7984
	16	1.5	0.6495
	4	1	1.2010
Huh7	8	2	0.8321
	16	3	0.6883

3