

The anti-tumor activities of Neferine on cell invasion and oxaliplatin sensitivity regulated by EMT via Snail signaling in hepatocellular carcinoma

Author name: Ganlu Deng ^{1,2}, Shan Zeng ^{1,2,3}, Junli Ma ^{2,3}, Yan Zhang ^{1,2}, Yanling Qu^{1,2},
Ying Han ^{1,2}, Ling Yin ^{1,2}, Changjing Cai ^{1,2}, Cao Guo ^{1,3}, Hong Shen ^{1,2,3} *.

Author's affiliation: 1 Institute of Medical Sciences, 2 Department of Oncology, 3 Key
Laboratory for Molecular Radiation Oncology of Hunan Province, Xiangya Hospital,
Central South University, Changsha, Hunan, China 410008

* Address reprint requests to: Dr. Hong Shen,
Institute of Medical Sciences, Xiangya Hospital,
Central South University, Changsha, Hunan, China 410008.
Tel: (86) 731-8432-7633
E-mail: hongshen2000@csu.edu.cn

Supplementary Information includes:
Supplementary Figure S1-S5.

Figure S1

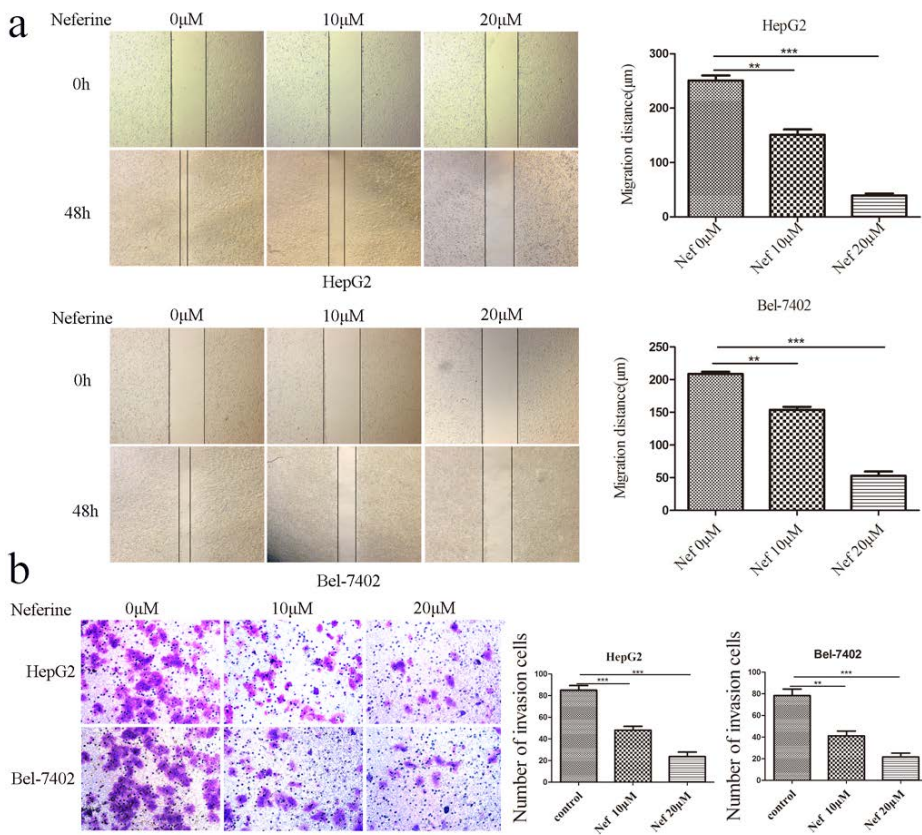


Figure S1: HCC cells migration, invasion ability inhibited by Neferine alone in vitro. (a) Migration ability tested by wound healing assay in HepG2 and Bel-7402 cells treated with Neferine at different concentrations. (b) Invasion ability examined by transwell assay in HepG2 and Bel-7402 cells treated with Neferine. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

Figure S2

Figure S2: The suppression effects of Neferine on EMT in HCC cells. (a & b) mRNA and protein expression of epithelial marker (E-cadherin), mesenchymal markers (N-cadherin & Vimentin), and EMT promoting transcription factor (Snail), which was determined by qRT-PCR and by Western blot in HCC cells treated with Neferine at different concentrations. (c) Representative double immunofluorescence staining for expression and co-localization of E-cadherin and Vimentin in HCC (Original magnification: $\times 400$).

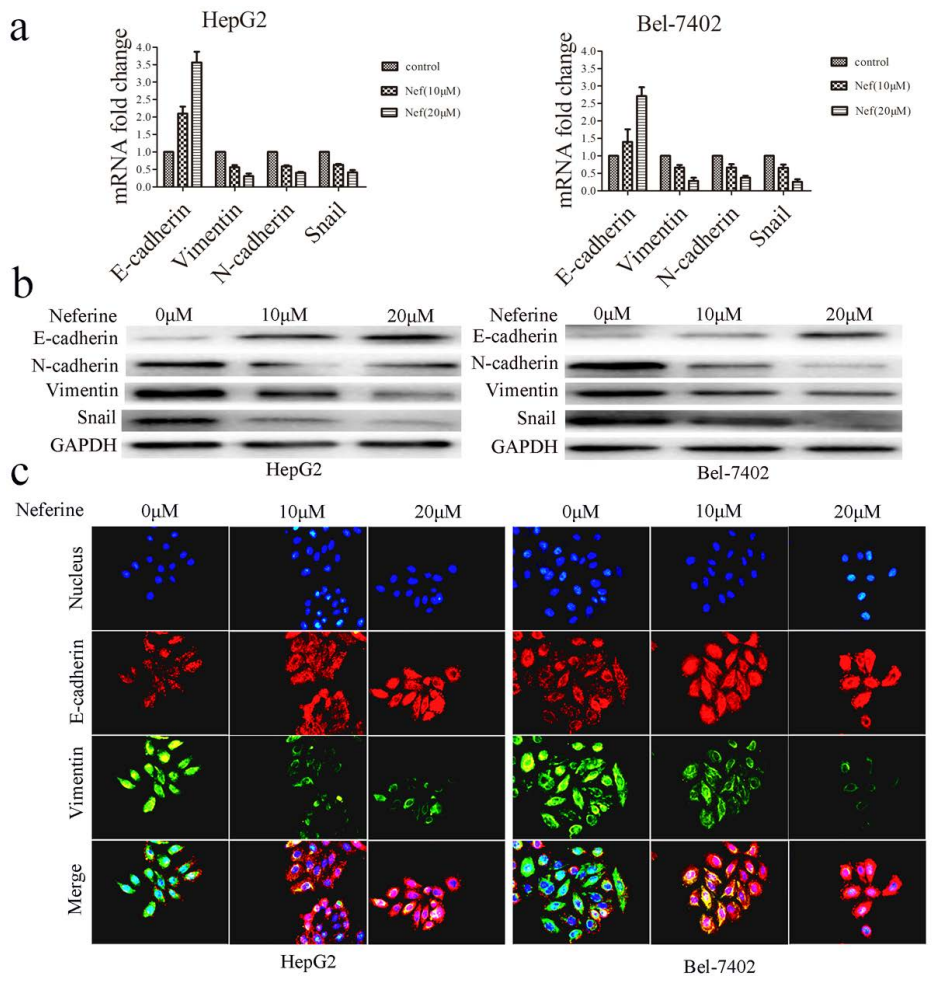


Figure S3

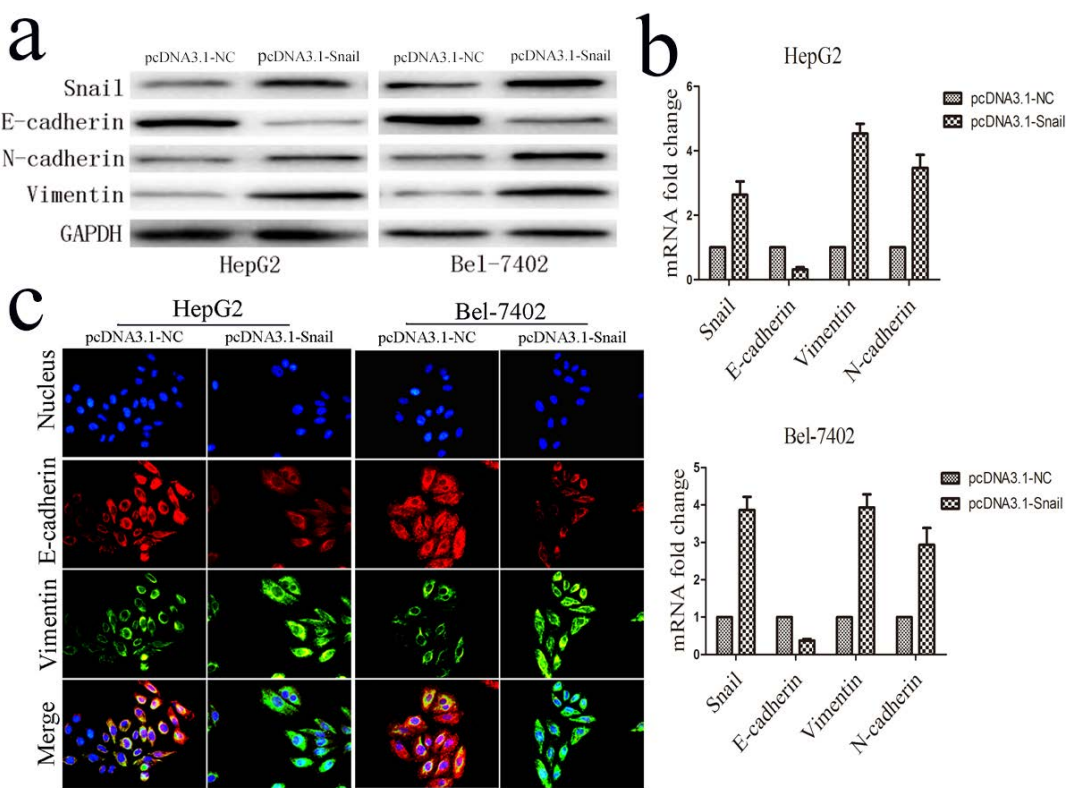


Figure S3: Snail overexpression promoted HCC EMT. (a & b) HCC EMT phenotype enhanced by Snail overexpression via increasing expression of mesenchymal markers and decreasing expression of epithelial marker. HepG2 and Bel-7402 cells were transfected by pcDNA3.1-Snail and pcDNA3.1-NC vectors for 48 hrs, respectively. (c) Expression and co-localization of E-cadherin and Vimentin protein in HepG2 and Bel-7402 cells regulated by overexpressed Snail, which was imaged by double immunofluorescence staining (Original magnification: $\times 400$).

Figure S4

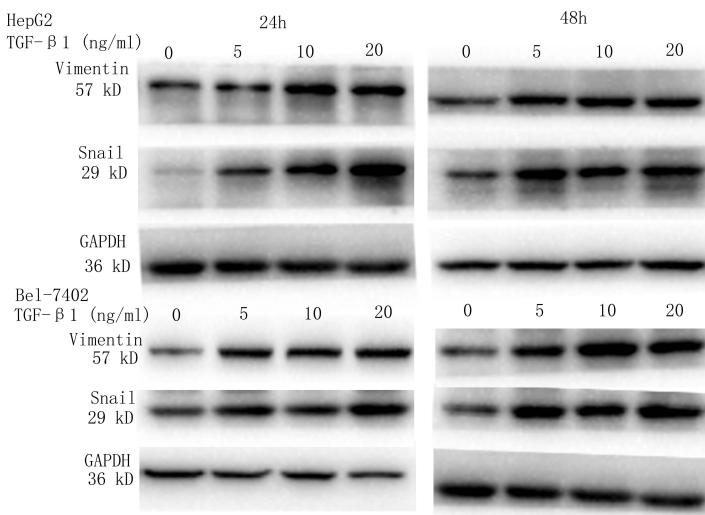


Figure S4: Original blots of high-contrast blots of Figure 3b. TGF- $\beta 1$ induced EMT progress. HCC cells were treated with 5 ng/ml, 10 ng/ml, 20 ng/ml TGF- $\beta 1$ for 24 hrs or 48 hrs to induce EMT. EMT biomarkers and EMT promoting transcription factor was determined by Western blot.

Figure S5

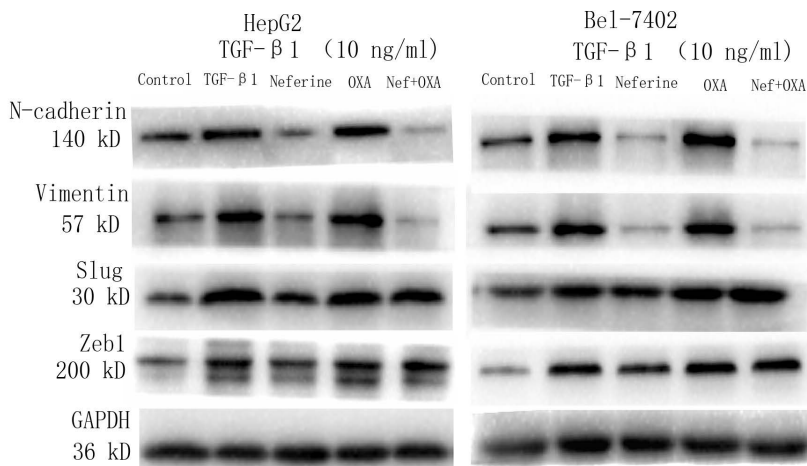


Figure S5: Original blots of high-contrast blots of Figure 4b. The suppression effects of Neferine on TGF- $\beta 1$ -induced EMT in HCC cells. Protein expression of EMT markers and EMT promoting transcription factors were determined Western blot in TGF- $\beta 1$ -induced HCC cells treated with Neferine and/or OXA.