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Vincosamide suppresses malignant behaviors of hepatoma cells by activating caspase-3 activity and blocking the PI3K/AKT signaling pathway

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Background: *Vincosamide* (Vinco) was first identified in the methanolic extract of the leaves of *Psychotria leiocarpa* and Vinco has important anti-inflammatory effects and activity against cholinesterase, Vinco also has a trait to ant-tumor. However, whether Vinco can inhibit the malignant behaviors of hepatocellular carcinoma(HCC) cells is still unclear. In the present study, we explored the role of Vinco in suppressing the malignant behaviors of HCC cells.

Methods: Microculture Tetrazolium Assay (MTT), trypan blue exclusion assay, the Cell Counting Kit (CCK)-8 and flow cytometric analysis were applied to detect the proliferation and death of HCC cells; electron microscopy was performed to observe the change in cellular mitochondrial morphology; scratch repair and Transwell assays were used to analyze the migration and invasion of HCC cells; expression and localization of proteins were detected by laser confocal microscopy and Western blotting; the growth of the cancer cells *in vivo* was assessed in a mouse tumorous model.

Results: At a dose of 10-80 μ g/ml, Vinco inhibited the proliferation, migration, and invasion and promoted apoptosis of HCC cells in a dose-independent manner, but had a low cytotoxicity effect on normal liver cells. Silenced expression of alpha-fetoprotein (AFP) could promote Vinco inhibits the proliferation of HCC cells. Additionally, 80 μg/ml of Vinco could significantly disrupt the morphology of mitochondria, and suppress the migration and invasion of HCC cells. The growth of HCC cells in the animal tumorous model was significantly inhibited after treatment with Vinco(10 mg/kg/day) for 3 days. The results of the present study indicated that Vinco(10-80μg/ml) played a role in activating caspase-3, promoting the expression of Phosphatase and tensin homolog (PTEN), and inhibiting the phosphorylation of AKT(Ser473) and mTOR(Thr2448), Vinco also has a trait for suppressing the expression of C-X-C chemokine receptor type 4 (CXCR4), Src, Matrix metallopeptidase 9 (MMP9), epithelial cell adhesion molecule (EpCAM), Ras, Oct4 and cancer stem cell "stemness markers" CD133 and CD44 in HCC cells.

Conclusions: Vinco has a role in inhibiting the malignant phenotype (behaviors) of HCC cells; the role molecular mechanism of Vinco may be involved in the restraining the expression of the growth-, metastasis-related factors Src, Ras, MMP9, EpCAM, CXCR4, and "stemness markers" CD133 and CD44; and activating the activity of caspase-3 and blocking PI3K/AKT signaling pathway. Thus, Vinco is available for chemotherapy to HCC patients.

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