

Evodiamine Illuminating New Horizons in Colorectal Cancer Therapy by Disrupting Hypoxia-Driven Angiogenesis via the HIF-1 α /VEGF Axis

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INTRODUCTION & AIM

As reported by GLOBOCAN 2022, colorectal cancer (CRC) is the second leading cause of cancer-related death, with more than 1.9 million new cases and approximately 900,000 deaths estimated in 2020 [1]. A hypoxic microenvironment is one of the most important characteristics of solid tumors. Hypoxia usually occurs when the tumor grows fast, and the newly generated vessels are insufficient to provide enough oxygen for the tumor growth. The anti-angiogenic therapy may also aggravate tumor hypoxia because of excessive inhibition of neovascularization. Hypoxia not only promotes the migration and invasion of tumor cells but also causes the upregulation of angiogenic factors, such as VEGF, which could stimulate the formation of tumor vessels [2]. Therefore, the agents targeting hypoxia may be good candidates for CRC treatment. Natural products are a crucial source for drug discovery and development because of their safety, efficacy, and immediate availability. Numerous drugs are isolated or innovated from natural product lead compound and more than 50% of drugs in the clinic are derived from natural products or their derivatives [2,3]. Evodiamine (EVD) is a natural alkaloid compound derived from the fruit of *Evodiae fructus*, and is mainly used for treating diarrhoea and headaches, while displaying anti-cancer potential against a wide range of cancers.

METHOD

Cell culture and viability assay. SW480 and Human umbilical vein endothelial cell (HUVEC) were taken from the Chinese Academy of Science in Hangzhou, China. HUVECs were cultured in RPMI 20%. RPMI-1640 medium, respectively, which contain 10% FBS and 1% penicillin/streptomycin. All the cells were cultured in a humidified atmosphere at 37 °C containing 5% CO₂. Tube formation assay. The 12-well plate was coated with 40 μ L-Matrigel and incubated for 1 h at 37 °C. HUVECs and HMEC-1 cells were harvested after indicated treatment in hypoxia incubator and then seeded into Matrigel-coated 96-well plate at a density of 2.5×10^4 per well. After another 6 h of incubation, the capillary tube structures were observed and captured with an inverted microscope (Olympus, Germany). The tube numbers were quantified as mean relative tube numbers of five random microscopic fields using ImageJ software. Western blotting assay and RT-PCR. The Western blotting assay was performed as previously described [2]. And for quantification, the ImageJ software was used. In Quantitative reverse transcription-polymerase chain reaction (RTqPCR) analysis The cells were pre-treated with or without EVD for 24 h and then cultured in a hypoxic environment for another 24 h. Statistical analysis. Quantitative data are presented as the mean \pm SEM. GraphPad Prism 5.0 was used to data analysis. Significance of differences among multiple groups was determined by one-way ANOVA followed by a Tukey's test. And $p < 0.05$ was assumed as a significant difference.

RESULTS & DISCUSSION

3.1. VEGF and HIF-1 α attenuate the development of colorectal cancer. As HIF-1 α is overexpressed during hypoxia, we confirm the VEGF expression in CRC by GEPIA (<http://gepia.cancer-pku.cn/>). VEGF is significantly high in CRC (Fig 1A) and is highly expressed in the 4th stage of the CRC (Fig. 1B). The overall survival is also influenced by VEGF and HIF-1 α (Fig. 1C). We further investigated and checked the correlation of VEGFA and HIF-1 α by the GEPIA database, it was confirmed that it significantly correlation in CRC (Fig. 1D). Upon hypoxic conditions, the levels of VEGF and HIF-1 α mRNA Expression were significantly upregulated compared with those under normoxic conditions, as we have examined the expression at different time periods (Fig 1E).

3.2. EVD Inhibited Hypoxia-Induced VEGF and HIF-1 α Expression in CRC Cells and Cell-Induced Angiogenesis Under Hypoxic Conditions. HIF-1 α accumulates under hypoxic conditions and is associated with tumour progression. Figure 1F showed the structure of Evodiamine. To investigate whether EVD could inhibit the growth of the CRC cells. We first evaluated the cytotoxicity with the MTT assay; EVD significantly inhibited the cell proliferation of CRC cells at various concentrations (IC₅₀, 8.342 μ M) (Fig 1G). The elevation of VEGF and HIF-1 α mRNA and protein expression was partly reversed by EVD treatment when we treated CRC cells with EVD at 2 μ M, 4 μ M and 8 μ M concentrations in the presence of hypoxia. EVD significantly inhibited VEGFA and HIF-1 α expression in CRC cells (Fig 2A, B and C). In order to explore the mechanisms of promoting tube formation under hypoxic conditions, we performed a tube formation assay under hypoxic conditions. Under hypoxic conditions, EVD significantly inhibit the tube formation of HUVEC cells ($p > 0.05$) (Figure 2D, E). However, HUVEC cells under hypoxic conditions significantly increased the tube formation.

3.3. EVD Inhibited HIF-1 α Accumulation and Activity in CRC Cells under Hypoxic Conditions. To explore the molecular target of EVD on hypoxia-induced angiogenesis. The effect of EVD in CRC cells *in vivo* was observed in nude mice using a xenograft tumor modal. CRC cells were implanted subcutaneously, and mice were treated with EVD or vehicle (control group (Ctrl), the low-dose EVD group, and the high-dose EVD group for a continuous 10 days. EVD administration significantly impedes tumor development in a dose-dependent, as seen in tumor weight and volume in comparison with the vehicle group (Fig. 2F, G). EVD treatment significantly impedes tumor development relative to the vehicle-treated group. No significant losses were observed in body and organ weight of the experimental mice. Concurrently, to evaluate the adverse effects of ADG after *in vivo* injection, Blood and different organs were collected from ADG-treated mice. No significant weight changes were observed in the Vital organs between the ADG-treated group and the control group, suggesting the absence of substantial toxicity. Additionally, we investigated the proliferation, and it was found that EVD can inhibit the proliferation of CRC tumor cells *in vivo* (Fig. 2H). Further examination on the molecular level in underway, and it was seen that EVD is a promising candidate for CRC inhibition.

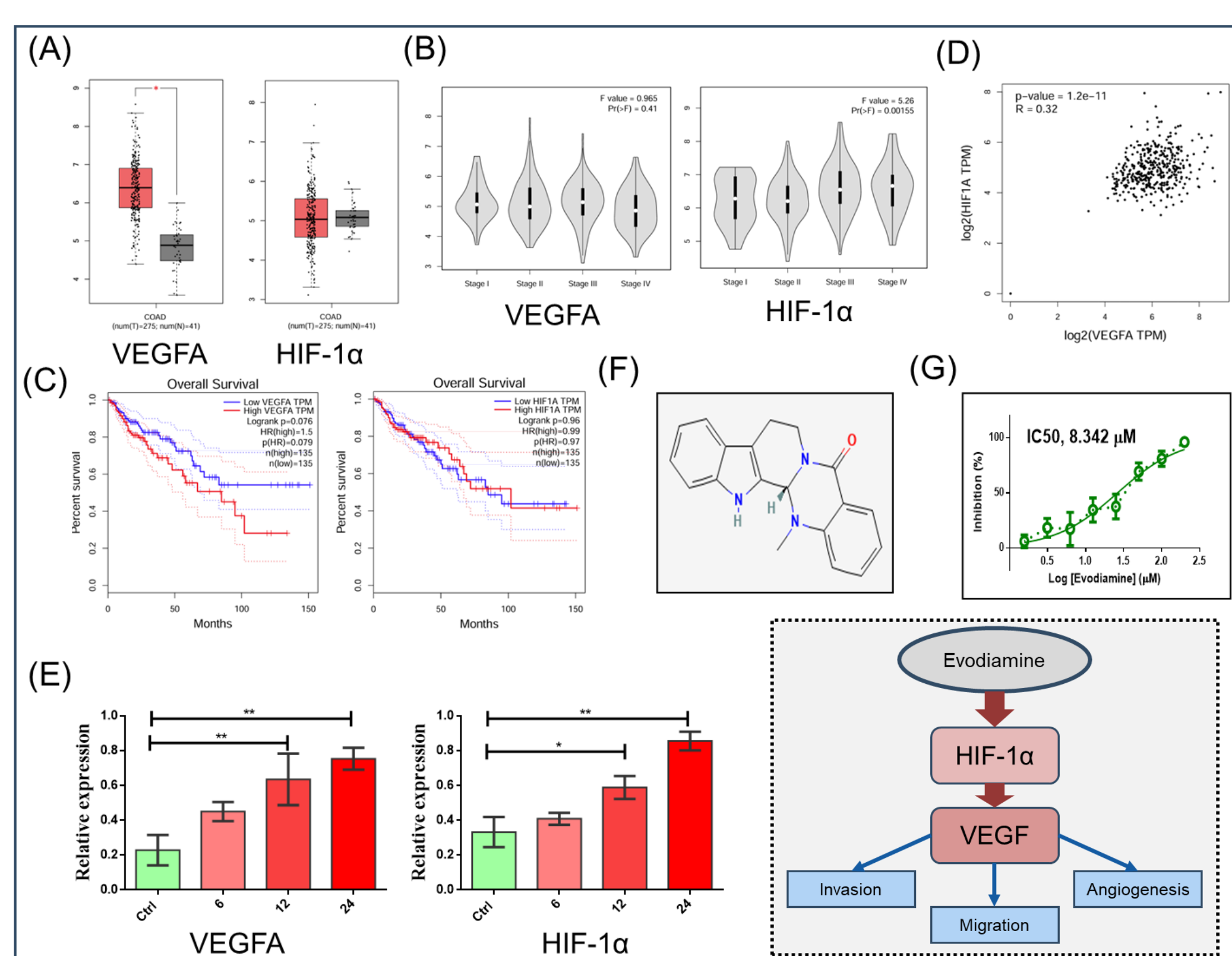


Figure 1: High expression is correlated with poor prognosis. (A) Pan-cancer gene analysis of VEGFA and HIF-1 α (GEPIA). (B) Stage gene analysis of VEGFA and HIF-1 α (GEPIA). (C) Overall survival gene analysis of VEGFA and HIF-1 α (GEPIA). (D) Correlation analysis of VEGFA and HIF-1 α (GEPIA). (E) Structure of Evodiamine. (F) Cell proliferation influence of EVD in COAD cells was analyzed by MTT assay at various concentrations for 48 h (n = 3). (G) Relative gene expression of VEGFA and HIF-1 α treatment with EVD in hypoxia and normoxia. Quantitative data were presented as mean \pm SD. * $p < 0.05$, ** $p < 0.01$.

Figure 3: Schematic diagram of Evodiamine inhibiting CRC via HIF-1 α and VEGF

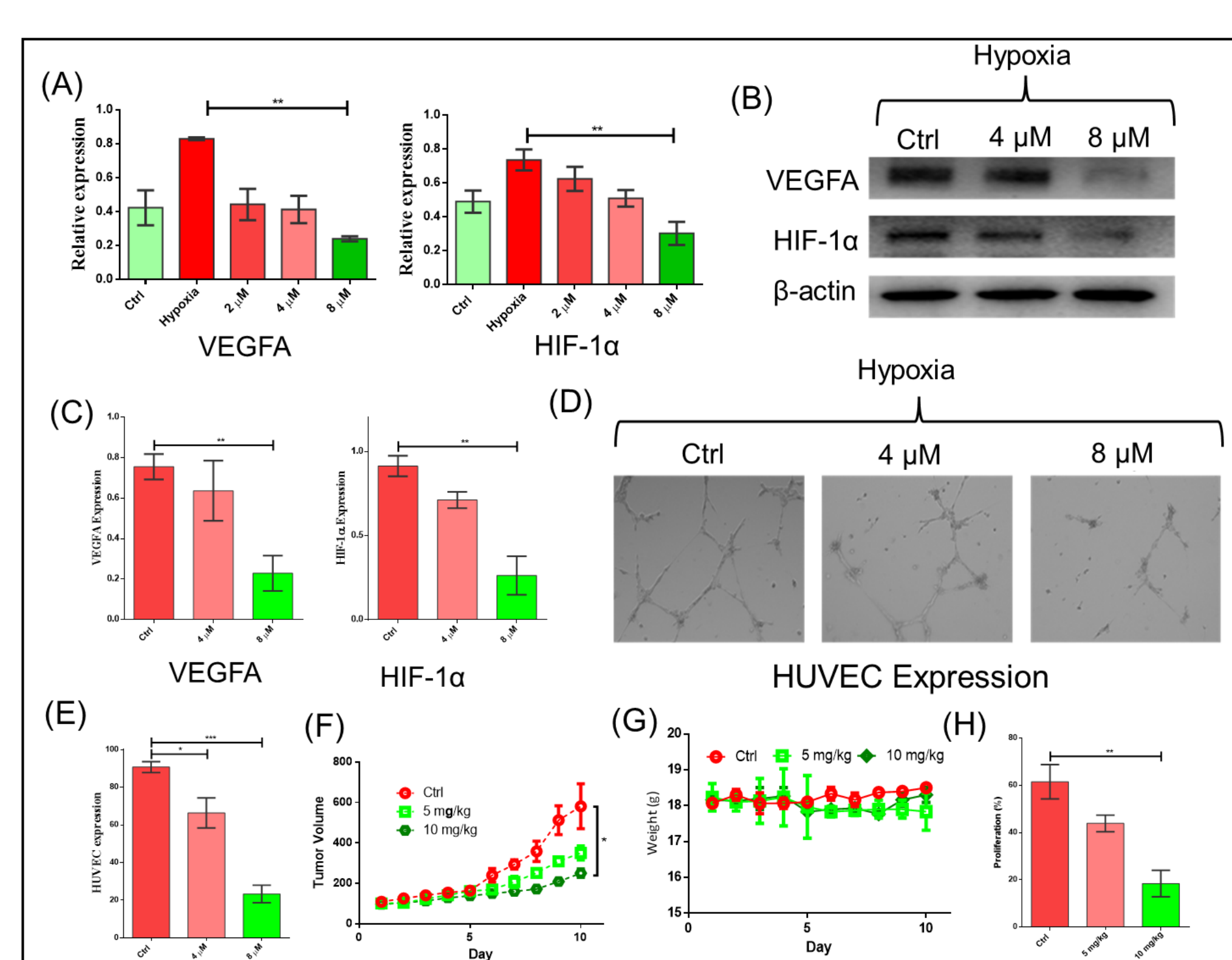


Figure 2: Evodiamine inhibits tumor formation *in vitro* and *in vivo*. (A) Relative gene expression of VEGFA and HIF-1 α treated with EVD at various concentrations in hypoxia and without hypoxia. (B) WB expression of VEGF and HIF-1 α in the presence of Hypoxia with EVD treatment. (C) Quantification of Figure B. (D) HUVEC cell expression Treated with EVD in the presence of hypoxia. (E) Quantification of Figure D. (F) Tumor volume in xenograft tumor-derived model from cells treated with EVD. (G) Weight of mice in xenograft tumor-derived model from treated and control groups. (H) Quantification of immunohistostaining of Ki67 in xenograft tumor-derived model (n = 3). Quantitative data were presented as mean \pm SD. * $p < 0.05$, ** $p < 0.01$.

CONCLUSIONS

In conclusion, Colorectal cancer remains a global health challenge, with tumor hypoxia driving angiogenesis, metastasis, and therapeutic resistance. Our study reveals that EVD, a natural compound traditionally used in leukemia treatment, holds significant promise as an anti-angiogenic agent in CRC. Through a series of *in vitro* assays, we demonstrated that EVD effectively inhibits hypoxia-induced angiogenesis, Reduces HIF-1 α accumulation and activity, and Impairs CRC cell migration and colony formation under hypoxic conditions. These findings underscore EVD's dual mechanism of action, targeting both HIF-1 α degradation and transcriptional suppression to disrupt the hypoxic tumor microenvironment. By inhibiting critical pathways driving angiogenesis and tumor growth, EVD emerges as a novel therapeutic candidate for CRC, particularly in advanced stages where hypoxia is prevalent.

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FUTURE WORK/ REFERENCES/ACKNOWLEDGMENT

While our study demonstrates the promising anti-angiogenic and anti-tumor effects of EVD in CRC under hypoxic conditions, several avenues warrant further exploration: Explore the role of other hypoxia-related factors (e.g., HIF-2 α , PHDs) in EVD-mediated angiogenesis inhibition. Explore structural modifications of EVD to improve its bioavailability, reduce toxicity, and enhance targeting of hypoxic tumor microenvironments. We extend our deepest gratitude to all who contributed to the success of this study. We acknowledge the use of core facilities at ZJUT, including the hypoxia incubator, microscopy, and molecular biology labs.