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4′-hydroxywogonin inhibits colorectal cancer angiogenesis by disrupting PI3K/AKT signaling

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ABSTRACT

Angiogenesis is fundamental for solid tumor growth and metastasis, and anti-angiogenic therapy has been an important therapeutic option for cancer treatment. Colorectal cancer (CRC) represents the fourth leading cause of cancer-related death worldwide. The current studies were aimed at investigating the anti-angiogenic effects of the natural compound 4′-hydroxywogonin (4′-HW) on CRC-related angiogenesis. Human CRC cell line SW620 cells and normal human intestinal epithelial HIEC cells were cultured and treated with interleukin-6 to mimic the tumor inflammatory microenvironment. Our data showed that 4′-HW reduced the viability of SW620 cells in a concentration- and time-dependent manner. 4′-HW also suppressed the proliferation of SW620 cells, but had little effect on the viability of HIEC cells. Moreover, 4′-HW concentration-dependently decreased the mRNA and protein expression of vascular endothelial growth factor-A (VEGF-A), the predominant pro-angiogenic cytokine in tumor angiogenesis. Subsequently, 4′-HW concentration-dependently inhibited the phosphorylation of phosphatidylinositol 3-kinase (PI3K) and AKT. PI3K inhibitor wortmannin, similar to 4′-HW, significantly downregulated the VEGF-A expression in SW620 cells, and combination of wortmannin and 4′-HW produced more significant effects. Finally, human umbilical vein endothelial cells (HUVECs) incubated with the conditioned medium of 4′-HW-treated SW620 cells exhibited impaired angiogenic capacity at Matrigel. Incubation with the neutralizing antibody against VEGF-Aalone also suppressed the angiogenic properties of HUVECs *in vitro*. Collectively, 4′-HW decreased the viability and reduced angiogenesis in CRC, which was associated with downregulation of VEGF-A expression by disrupting the PI3K/AKT pathway. Our discoveries suggested 4′-HW as a promising anticancer agent against CRC targeting angiogenesis.

1. Introduction

Angiogenesis can have a major effect on the formation and progression of solid tumordue to the requirement of new vessels to supply the blood with oxygen and nutrients. Usually, when the tumor diameter exceedstwo millimeters, its growth cannot be sustained by tissue penetration any more, leading to hypoxic microenvironment [1]. Under this condition, tumor cells express and secrete a variety of pro-angiogenic cytokines, which bind to their specific receptors on endothelial cells, the inner layer of existing vessels. Accordingly, endothelial cells are activated, and sprout by proliferation and migration [2]. It has been generally acknowledged that vascular endothelial growth factor-A (VEGF-A) is the predominant pro-angiogenic cytokine in tumor angiogenesis. Cancer cells secrete VEGF-A to facilitate angiogenesis even before a visible tumor is formed [3]. Upon binding to VEGF-A, the VEGF receptor 2 undergoes phosphorylation, which

Abbreviations: 4′-HW, 4′-hydroxywogonin; PI3K, phosphatidylinositol 3 kinase; VEGF-A, vascular endothelial growth factor-A; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PTEN, phosphatase and tensin homology deleted on chromosome 10; mTOR, mammalian target of rapamycin; ELSIA, enzyme-linked immunosorbent assay

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triggersa series of downstream signaling events, promotingendothelial cell proliferation [4]. The expression of VEGF-A by cancer cells is regulated by multiple signaling pathways and transcriptionfactors. Activation of the phosphatidylinositol 3-kinase (PI3K)/AKTpathway can increaseVEGF-A expression and thereby promote angiogenesis in many types of tumors [5–7].

Colorectal cancer (CRC) represents the fourth leading cause of cancer-related deathall over the world. Despite recent advances in the treatment of CRC, the median overall survival time of patients with metastatic CRC is still less than 30 months, posing a serious threat to human physical and mental health [8]. It is therefore important to elucidate the molecular mechanism underlying CRC pathology so as to develop therapeutic strategies for CRC. Recently, increasing studies have demonstrated a critical role of aberrant angiogenesis in CRC. For example, the level of VEGF-A is elevated and correlate with a poor clinical outcome in patients with CRC, and thus is considered a negative prognostic indicator for CRC [9,10]. Several latest studies showed that CRC cells produce VEGF-A to facilitate angiogenesis and tumorigenesis [11–13]. Accordingly, anti-angiogenic therapy targeting VEGF-A has been proved to be one of the most crucial and promising approaches to limit CRC growth [14]. Wogonin, a naturally occurring mono-flavonoid compound, has been reported to suppress proliferation and induce cell cycle arrest in human CRC cells, showing therapeutic promising against CRC [15]. Interestingly, wogoninwas also found to inhibit tumor angiogenesis [16,17]and suppress hydrogen peroxide-induced angiogenesis in human umbilical vein endothelial cells (HUVECs) [18]. Based on these recognitions, we hypothesized that the structural derivatives ofwogonin could inhibit colorectal cancer angiogenesis. In the present study, we investigated the anti-angiogenic effects of 4′-hydroxywogonin (4′-HW, $C_{16}H_{12}O_6$, Fig. 1A), a common derivative of wogonin, on colorectal cancer angiogenesis, and explored the underlying signaling events.

2. Materials and methods

2.1. Reagents and antibodies

4′-HW(purity > 98%) was purchased from Shanghai Chembest Research Laboratories Limited (Shanghai, China). PI3K inhibitor wortmannin, and cell proliferation inhibitor mitomycinC were purchased from Selleck Chemicals (Houston, TX, USA). All of the above compounds were dissolved in dimethyl sulfoxide (DMSO), and DMSO was used alone as a vehicle control. Recombinant human IL-6 was purchased from Solarbio Life Science (Beijing, China). Primary antibodies used in Western blot assays against VEGF-A, p-PI3K^{Tyr467}, PI3K, *p*-AKT^{Thr308}, AKT, and GAPDH were all purchased from Proteintech Group (Chicago, IL, USA).

2.2. Cell culture

Human CRCSW620 cells, normal human intestinal epithelial HIEC cells, and HUVECs were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). These types of cells were maintained as monolayer culture in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS), 100 μg/ml penicillin and 100 μg/ml streptomycin, at 37 monolayer culture in Roswell Park M_2 atmosphere.

2.3. Cell viability assay

Cellswere seeded in 96-well plates (1×10^4/well) and cultured in RPMI 1640 medium supplemented with 10% FBS for 24 h. Cells were

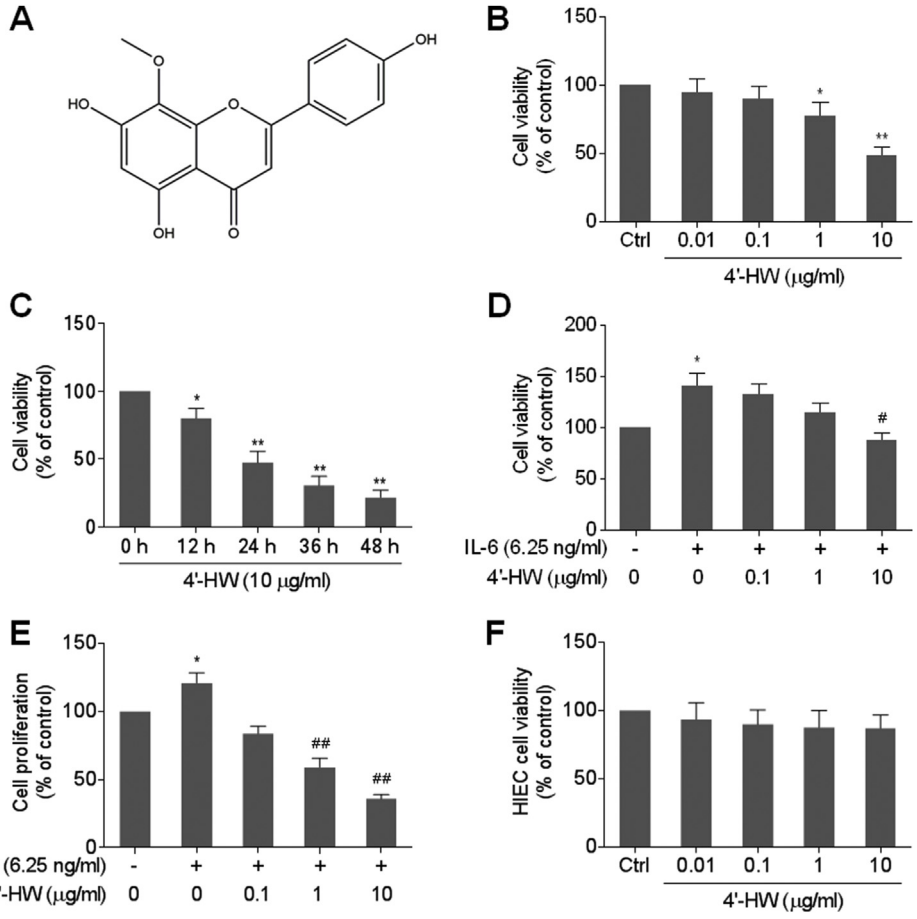

Fig. 1. 4′-HW in reduces the viability of SW620 cells treated with or without IL-6. SW620 cells (B–E) or HIEC cells (F) were treated with 4′-HW and/or IL-6 at indicated concentrations for 24 h or for indicated time duration. (A) Chemical structure of 4′-HW. (B-D, F) MTT assay for evaluating cell viability. Cell viability was expressed as percentage of control. Results were from triplicate experiments. Significance: $^*P < 0.05$ versus control, $^{**}P < 0.01$ versus control, $^{\#}P < 0.05$ versus IL-6. (E) BrdU incorporation assay for determining cell proliferation. Cell proliferation was expressed as percentage of control. Significance: $^*P < 0.05$ versus control, $^{\#\#}P < 0.01$ versus IL-6.

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