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Investigation of the anti-angiogenesis effects induced by deoxypodophyllotoxin-5-FU conjugate C069 against HUVE cells

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ABSTRACT

We have found that the deoxypodophyllotoxin-5-fluorouracil conjugate, 4′-*O*-demethyl-4-deoxyppodophyllotoxin-4′-yl 4-((6-(2-(5-fluorouracil-yl)acetamido) hexyl)amino)-4-oxobutanoate (C069), possessed superior cytotoxicities and less toxicity compared with etoposide. In this paper, the anti-angiogenic and vascular disrupting activities of C069 were examined with several in vitro and in vivo models. First, we demonstrated that C069 significantly inhibited the proliferation, migration, tube formation and disrupted the formed tube-like structures of HUVE cells, and inhibited angiogenesis in chicken chorioallantoic membrane assay. Furthermore, we found that C069 inhibited tube formation of HUVE cells by down-regulating the MMP-2, MMP-9, and phosphorylation of Akt and β-catenin. These results provided the initial evidence that C069 exerts potent anti-angiogenic and vascular disrupting effects.

Angiogenesis is a physiological process involving the formation of new blood vessels from pre-existing blood vessels. It is a complex process characterized by the proliferation, migration, sprouting and elongation of endothelial cells,[1] and plays an important role during cell reproduction and organ development, as well as in wound healing processes. Sustained angiogenesis is one of the central hallmarks of cancer and has been validated as a key target for cancer therapy.[2–4]

Endothelial cells secret matrix metalloproteases (MMPs) which belong to family of zinc-dependent proteases that can proteolytically digest specific extracellular matrix components. Endothelial cells invade into the surrounding matrix, creating space for a vascular lumen. Therefore, proteolysis of matrix components is required to initiate angiogenesis.[5,6] Several MMPs including MMP-2 and MMP-9 have been reported as crucial proteases for angiogenic switch.[7] The activities of MMP-2 and MMP-9 facilitate endothelial cell invasion, leading endothelial cell survival and/or migration and influence on release of pro-angiogenic factors or destruction of angiogenesis inhibitors.[8]

The β-catenin signaling pathway controls a vast array of biological process including cell proliferation, differentiation, apoptosis, migration, polarity establishment and stem cell self-renewal.[9] Abnormal β-catenin signaling leads to a diverse range of human diseases, such as Alzheimer's disease, polycystic kidney disease, osteoporosis, schizophrenia, metabolic disease, and various cancers.[10] AKT is activated downstream from epidermal growth factor receptor signaling, and phosphorylates β-catenin at Ser^{552} in vitro and in vivo. AKT mediated phosphorylation of β-catenin causes its disassociation from cell-cell contacts and accumulation in both the cytosol and the nucleus, and increases its transcriptional activity and promotes tumor cell invasion.[11]

Podophyllotoxin (PPT) shows strong cytotoxic activity against many tumor cell lines.[12,13] Several anti-cancer drugs, such as etoposide (VP-16) and teniposide (VM-26), have been yielded through the structural modifications to PPT.[14] In order to develop drugs based on natural products with efficient anticancer activities and low toxicity, we have selected different linkers to combine deoxypodophyllotoxin and 5-FU to provide series of novel hybrids.[15–17] In our previous publication,[18] we have found that 4′-*O*-demethyl-4-deoxypodophyllotoxin-4′-yl 4-((6-(2-(5-fluorouracil-yl) acetamido) hexyl)amino)-4-oxobutanoate (C069, Fig. 1) increased cytotoxic activity in cancer cells and decreased toxicity in non-cancerous cells compared with the clinical drug etoposide, and C069 also inhibits A549 cells migration by down-regulation of MMP-9 and up-regulation of TIMP-1. Progression from localized tumours to metastatic cancers usually involves the enhancement of tumor migration, invasion and angiogenesis properties.[3] Here we report the effects of C069 on antiproliferation, metastasis and angiogenesis in human umbilical vein endothelial cells (HUVECs).

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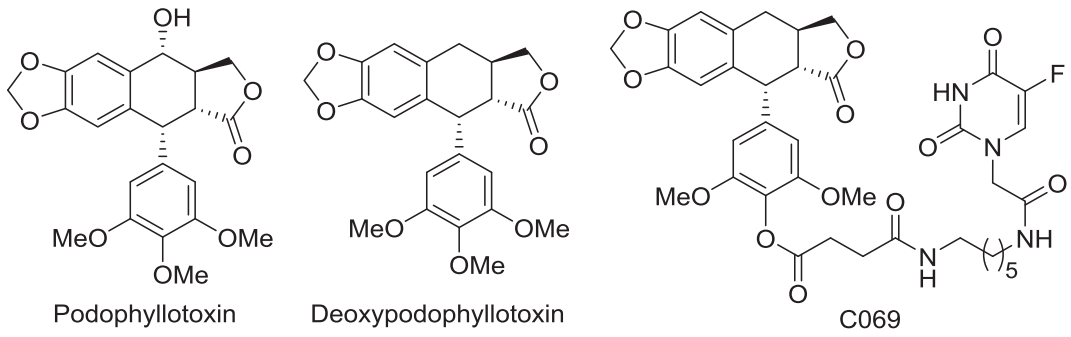

Figure 1. Chemical structures of podophyllotoxin, deoxypodophyllotoxin and C069.

The proliferation, migration, and tube formation of endothelial cells represent the three primary steps of angiogenesis.[19,20] The effects of C069 on HUVECs indicated that C069 abrogated angiogenesis in vitro. So we initially tested the effect of C069 on endothelial cell proliferation. To assess the anti-proliferation effect of C069, MTT assays were performed in HUVECs. As depicted in Fig. 2A, C069 treatment resulted in a dramatic, dose- and time-dependent inhibition of cell proliferation. In addition, this inhibition effect was lower at all concentrations of C069 (20, 50, and 100 μM) compared to VP-16 (30 μM) in normal, human WI-38 cells (Fig. 1B).

Endothelial cell migration occurs via chemotaxis, which is a key step in the process of angiogenesis.[21] The effects of C069 (0, 0.1 and 0.3 μM) on the chemotactic motility of HUVECs were assessed in a wound-healing migration assay. As shown in Fig. 3, C069 dramatically inhibited the wound-healing migration.

In an effort to further study the anti-angiogenic effect of C069, we were interested in in vitro tube formation assay using HUVECs

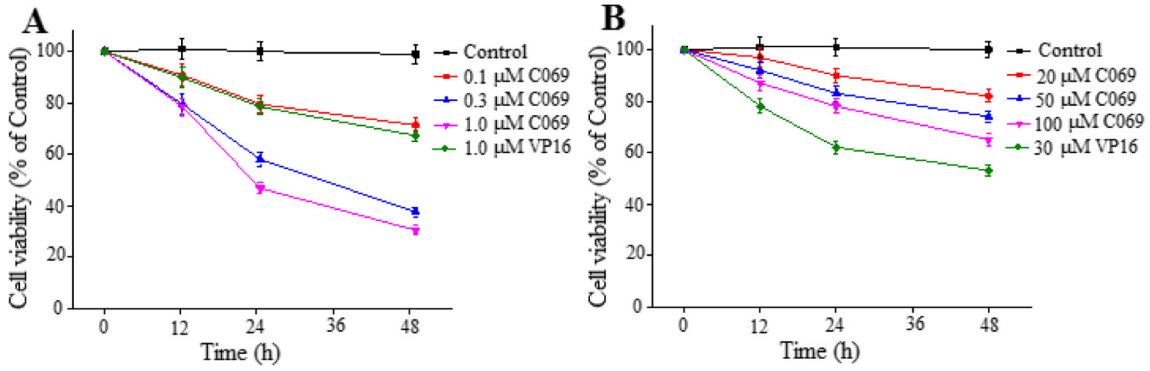

Figure 2. C069 inhibits the proliferation of HUVECs and WI-38. Survive curves of HUVECs (A) and WI-38 (B) in vitro were treated with C069 and reference compound VP-16 at different concentrations and times. Data were shown as the mean ± SD of three independent experiments.

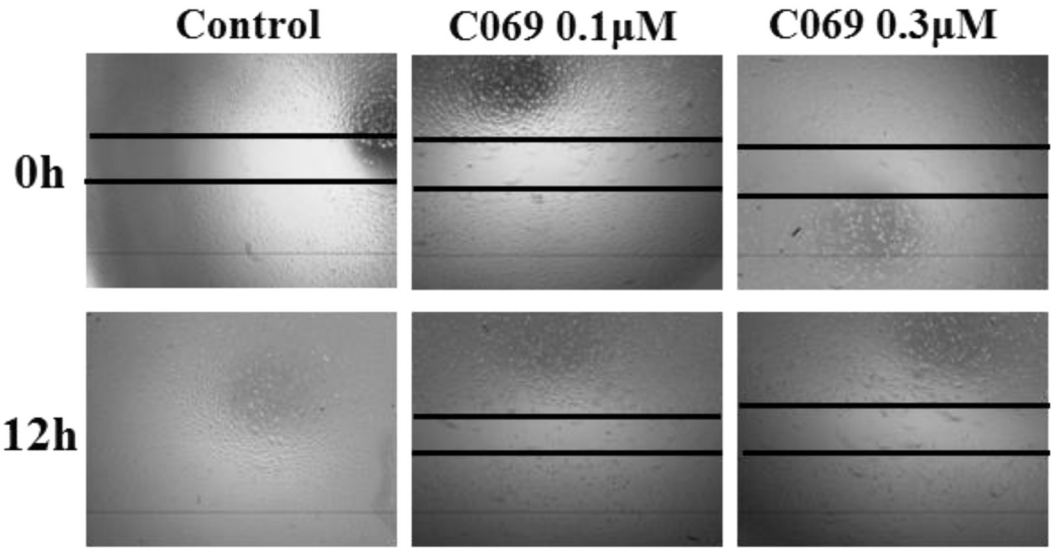

Figure 3. Effect of C069 on the migration of HUVECs cells. Representative images of HUVECs cells were treated with vehicle, 0.1 μM C069, 0.3 μM C069 for 12 h, respectively. Magnification: 40×; scale bar, 500 μm