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Assessment of the anti-metastatic properties of sanguiin H-6 in HUVECs and MDA-MB-231 human breast cancer cells

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ABSTRACT

The anti-metastatic properties of sanguiin H-6 were examined in human umbilical vein vascular endothelial cells (HUVECs) and MDA-MB-231 human breast cancer cells. In HUVECs, sanguiin H-6 inhibited the density of migrated cells compared to that observed after treatment with the vehicle. In addition, sanguiin H-6 at a concentration of 6.25 μM significantly blocked tube formation. Treatment with up to 25 μM sanguiin H-6 had no effect on MDA-MB-231 cells, whereas treatment with 200 μM sanguiin H-6 decreased cell viability. Sanguiin H-6 significantly decreased the expression levels of vascular endothelial growth factor (VEGF), phosphorylated Akt, and extracellular signal-regulated kinase 1/2 (ERK1/2) in MDA-MB-231 cells. These findings suggest that sanguiin H-6 is potentially useful as an anti-metastatic agent.

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Breast cancer is one of the major cancers affecting women worldwide.[1] Current therapeutic approaches for the treatment of human breast cancer include hormonal therapy, surgery, radiotherapy, hyperthermia therapy, and chemotherapy.[2] However, conventional strategies for the treatment of breast cancer remain unsatisfactory and limited, mainly due to the adverse effects of metastases rather than the primary tumor itself. In the case of breast cancer, most patients with advanced disease develop lung and bone metastasis, which are common causes of morbidity and sometimes mortality.[3]

The process of cancer metastasis is closely related to angiogenesis.[4] Angiogenesis is the formation of new blood vessels from pre-existing vasculature. It is a multistep process that requires at least four independent events mediated by endothelial cells, including detachment from basement membranes, proliferation, migration, and maturation.[5,6] Endothelial cells, a major cell component of endothelium that forms the inner lining of a blood vessel, play a critical role in angiogenesis. Proliferation, migration, and the formation of tube-like structures in cultured endothelial cells are typical characteristics noted in in vitro angiogenesis assays.[7] Vascular endothelial growth factors (VEGFs) are crucial regulators of vascular development during embryogenesis (vasculogenesis) as well as blood-vessel formation (angiogenesis) in adults.[8] In angiogenesis, phosphoinositide 3-kinase (PI3K)/Akt signaling is activated and phosphorylated Akt, which is the activated form of Akt, induces the expression of tumor growth related proteins and angiogenic factors.[9]

Sanguiin H-6 (Fig. 1A) is a polyphenol compound categorized as an ellagitannin and is found in *Rosaceae* (great), Rubus species (strawberries, red raspberries and cloudberries), and the crude drug *Sanguisorbae Radix*.[10,11] It is dimer of casuarictin linked by a bond between the gallic acid residue and hexahydroxydiphenic acid units.[11] It is known that ellagitannin is poorly bioavailable, and eventually break down into ellagic acid upon entering the small intestine.[12] Sanguiin H-6 possesses antioxidant activity, demonstrates amylase inhibition, and shows resistance to powdery mildew.[11,12] Among the known sanguiin H analogues, sanguiin H-6 only blocked the binding of $VEGF_{165}$ to KDR/Flk-1 and reduced the VEGF and bFGF-induced HUVECs proliferation.[13] Sanguiin H6 also inhibited TGF-β1-induced Smad signaling and EMT development in A549 lung cancer cells.[10] In the present study, we aimed to assess the effect of sanguiin H-6 on the angiogenesis process in HUVECs and MDA-MB-231 cells by conducting cell-based experiments.

HUVECs are one of the most frequently used in vitro angiogenesis models.[5,7] The percentage cell viability of HUVECs (ATCC, Manassas, VA, USA) after treatment with sanguiin H-6 is shown

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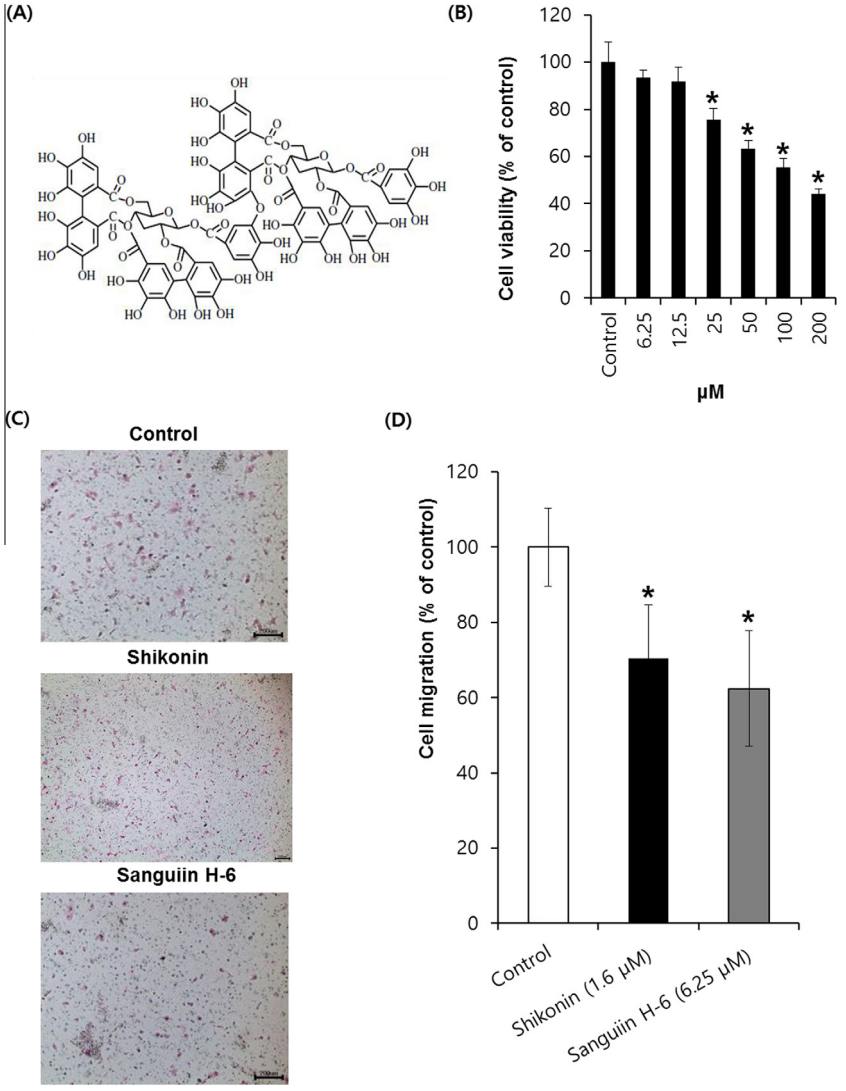

Figure 1. Effects of sanguiin H-6 on HUVEC proliferation and assessment of its cytotoxicity. (A) Structure of sanguiin H-6. (B) Cells were treated with sanguiin H-6 at a series of concentrations (6.25–200 μM) or DMSO vehicle (control) for 24 h, and cell viability was then evaluated by the MTT assay. Effect of sanguiin H-6 on HUVEC migration. (C) Photographs of migration of HUVECs on Matrigel after incubation with shikonin and sanguiin H-6. (D) The relative quantities of migrated cells. $p < 0.05$ compared to the control value.

in Figure 1B; sanguiin H-6 inhibited HUVEC proliferation in a dose-dependent manner. Sanguiin H-6 was isolated from red raspberries by using Sephadex LH-20 column chromatography and preparative HPLC (Supplementary data). Treatment with up to 12.5 μM of sanguiin H-6 had no effect on the HUVECs, whereas treatment with 25–200 μM decreased cell viability. Consequently, we propose that sanguiin H-6 exerts growth-inhibitory effects on HUVECs, but without causing significant cytotoxicity up to 12.5 μM. Cell migration is essential to the angiogenesis response, so we assessed the effect of sanguiin H-6 on HUVEC migration. Shikonin was used as positive control compound.[14] Non-toxic doses of sanguiin H-6 (6.25 and 12.5 μM) were selected for further experiments. Both concentrations of sanguiin H-6 (6.25 and 12.5 μM) exerted similar effects and the result for lower concentration is shown in Figure 1C and D. As shown in Figure 1C, the density of migrated cells after treatment with shikonin and sanguiin H-6 was significantly lower than that after treatment with the control. The percentage inhibition of migration of sanguiin H-6-treated cells was 37.6% of that observed in the control group (Fig. 1D).

Next, a tube formation assay was performed to investigate the morphogenic potential of sanguiin H-6. The effect of a nontoxic dose (6.25 μM) of sanguiin H-6 on HUVEC tube formation is shown in Figure 2. Representative tube images are shown in Figure 2A, and sanguiin H-6 at a concentration of 6.25 μM significantly blocked tube formation (Fig. 2B). Compared with control, significant inhibition of tube formation of 41.5% was observed with sanguiin H-6 at 6.25 μM.

The anti-metastatic effect of sanguiin H-6 was further assessed using human breast cancer MDA-MB-231 cells (ATCC, HTB-26) that are extremely invasive triple-negative breast cancer cells, which are resistant to several anticancer agents.[15] Therefore, MDA-MB-231 cells provide an ideal in vitro model to analyze the effects of anti-metastatic agents. The effects of sanguiin H-6 on the viability of MDA-MB-231 cells were investigated by treating the cells with increasing concentrations of sanguiin H-6 (6.25–200 μM) for 24 h (Fig. 3A). Treatment with up to 25 μM sanguiin H-6 had no effect on MDA-MB-231 cells, whereas treatment with 200 μM sanguiin H-6 decreased cell viability.

Angiogenesis is regulated by highly coordinated processes in which multiple angiogenic factors are involved. Given the complexity of these signals, it is remarkable that a single growth factor, VEGF, acts as a major regulator of angiogenesis.[16] PI3K/Akt

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