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Raddeanin A, a triterpenoid saponin isolated from *Anemone raddeana*, suppresses the angiogenesis and growth of human colorectal tumor by inhibiting VEGFR2 signaling

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ABSTRACT

Raddeanin A (RA) is an active triterpenoid saponin from a traditional Chinese medicinal herb, *Anemone raddeana* Regel. It was previously reported that RA possessed attractive antitumor activity through inhibiting proliferation and inducing apoptosis of multiple cancer cells. However, whether RA can inhibit angiogenesis, an essential step in cancer development, remains unknown. In this study, we found that RA could significantly inhibit human umbilical vein endothelial cell (HUVEC) proliferation, motility, migration, and tube formation. RA also dramatically reduced angiogenesis in chick embryo chorioallantoic membrane (CAM), restrained the trunk angiogenesis in zebrafish, and suppressed angiogenesis and growth of human HCT-15 colorectal cancer xenograft in mice. Western blot assay showed that RA suppressed VEGF-induced phosphorylation of VEGFR2 and its downstream protein kinases including PLCγ1, JAK2, FAK, Src, and Akt. Molecular docking simulation indicated that RA formed hydrogen bonds and hydrophobic interactions within the ATP binding pocket of VEGFR2 kinase domain. Our study firstly provides the evidence that RA has high antiangiogenic potency and explores its molecular basis, demonstrating that RA is a potential agent or lead candidate for antiangiogenic cancer therapy.

Introduction

Raddeanin A (RA) (Fig. 1a) is an oleanane-type triterpenoid saponin extracted from the root of *Anemone raddeana* Regel, a traditional Chinese medicinal herb (Luan et al. 2013). Previous study showed that RA exerted antitumor activity both *in vitro* and *in vivo*. RA inhibited proliferation and induced apoptosis of various human gastric cancer cell lines BGC-823, SGC-7901, MKN-28 and human non-small cell lung cancer H460 cells (Gao et al. 2010; Xue et al. 2013). Furthermore, RA exhibited antitumor efficacy in S180, H22 and U14 tumor xenograft in mice (Wang et al. 2008). However, it remains unknown whether RA can suppress angiogenesis, a crucial step in tumor development.

Angiogenesis, which involves multiple cells and soluble factors for the formation of new blood vessels from the preexisting ones, plays a pivotal role in the process of tumor growth and metastasis (Grothey and Galanis 2009). Blocking angiogenesis is a validated effective therapeutic approach against cancer, and several antiangiogenic agents (Avastin, Sutent, Nexavar, Votrient, Inlyta, Zaltrap, Stivarga, etc.) (Meadows and Hurwitz 2012; Mullard 2013) have been successfully translated into cancer clinic. Besides the approved monoclonal antibody and small-molecule tyrosine kinase inhibitors (TKIs), natural products from medical herb are currently attracting a growing amount of researchers to excavate their antiangiogenic activity (Song et al. 2012; Wang et al. 2013; Xu et al. 2013; Li et al. 2014).

The present study reveals the antiangiogenic potency of RA using human umbilical vein endothelial cell (HUVEC) (a classical *in vitro* cell model mimicking tumor vascular endothelial cells), chick chorioallantoic membrane (CAM) model, and transgenic zebrafish angiogenesis model. The tumor antiangiogenic efficacy of RA was evaluated in the subcutaneous HCT-15 xenograft mice model. Moreover, the antiangiogenic molecular mechanism of RA was explored by western blot and molecular docking assay.

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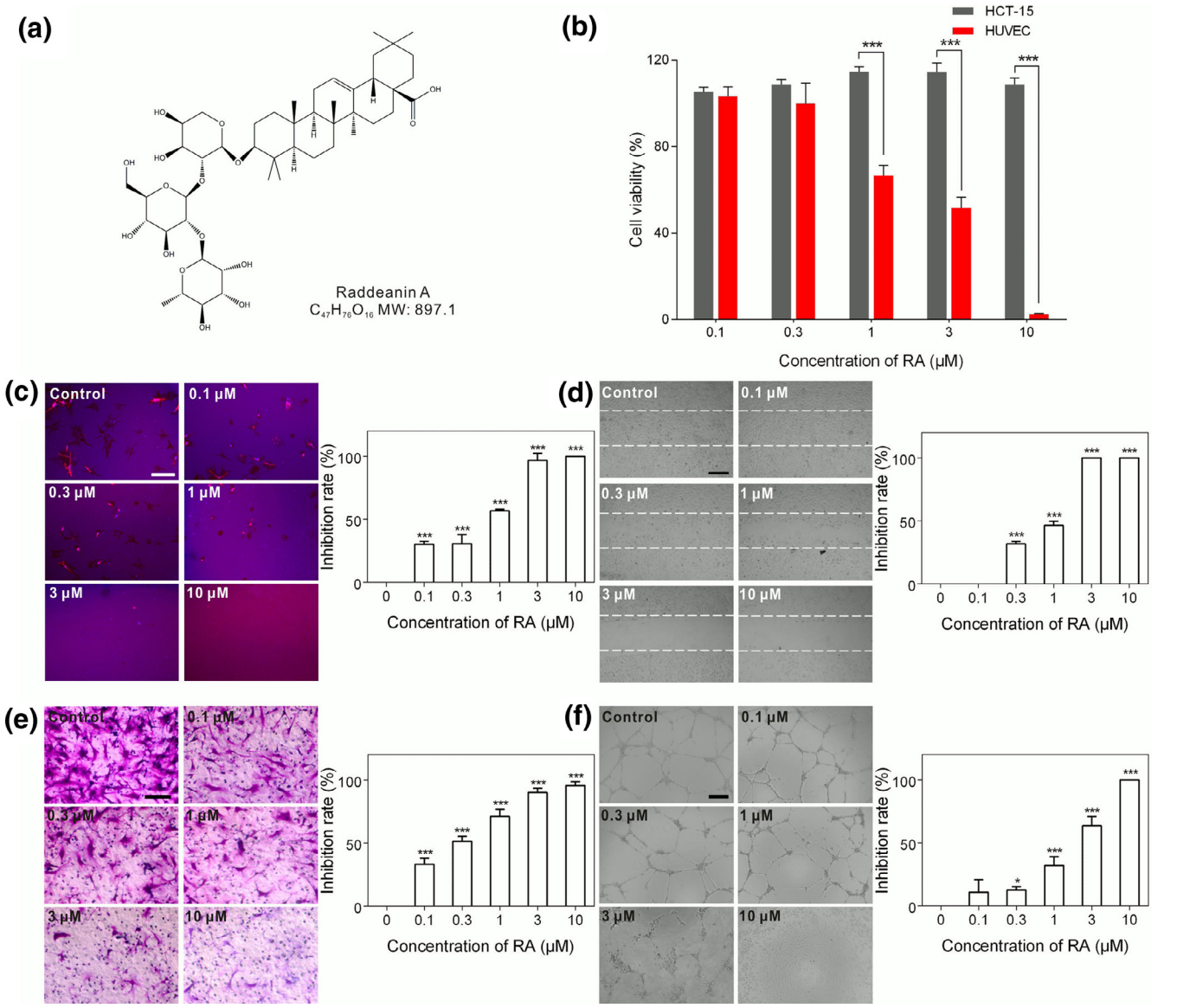

Fig. 1. RA more effectively inhibited the viability of HUVEC relative to HCT-15 cells, and it also significantly suppressed HUVEC motility, migration and tube formation. (a) Chemical structure of RA. (b) RA dose-dependently inhibited the viability of HUVEC, but not HCT-15 cells. Values are expressed as mean ± SD, $n = 4$. $^{***}p < 0.001$ as compared with HCT-15. (c) RA inhibited HUVEC motility. HUVEC treated with different concentration of RA were allowed to migrate on the blue fluorescent beads coated wells for 20 h, then the motion track area of HUVEC was analyzed using Thermo Scientific ArrayScan XTI High Content Analysis Reader. Bar, 200 μm. (d) RA inhibited HUVEC migration in wound healing assay. Dotted lines indicated the boundary of initial scraping. Bar, 500 μm. (e) RA suppresses HUVEC vertical migration in transwell assay. HUVEC migration after 8 h treatment of RA was assayed using Transwell Boyden chamber. The migrated cells were visualized by crystal violet and quantified using Image-Pro Plus 6.0 software. (f) RA inhibited tube formation of HUVEC. HUVEC were placed in the matrigel coated 96-well plate. After 10 h, the tubular structures were photographed. Representative photographs and quantitative analysis are shown in each panel. Values are expressed as mean ± SD, $n = 4$. $^{*}p < 0.05$, $^{***}p < 0.001$ as compared with control.

Materials and methods

Materials, cell lines and animals

RA was purchased from Pure-one Bio Technology Company (Shanghai, China). Recombinant human vascular endothelial growth factor ($VEGF_{165}$) was obtained from ProSpec-Tany Technogene Ltd. (Ness Ziona, Israel). Antibodies for western blotting were purchased from Cell Signaling Technology (Danvers, MA).

Primary human umbilical vascular endothelial cells (HUVEC) were obtained from Lifeline Cell Technology and cultured in completed endothelial cell medium (Lifeline Cell Technology, Frederick, MD). Human colorectal tumor cell line HCT-15 was obtained from the American Type Culture Collection (Manassas, VA) and cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and antibiotics (100 mg/ml of streptomycin and 100 U/ml of penicillin). Both HUVEC and HCT-15 were cultured at 37 °C in a humidified atmosphere containing 5% CO_2.

Zebrafishes (fli1a:EGFP transgenic line) were raised and maintained under standard conditions (Westerfield 1995). Embryos were staged according to the previous protocol (Kimmel et al. 1995). BALB/c nude mice were provided by Shanghai Laboratory Animal Center (Chinese Academy of Sciences, Shanghai, China) and housed in an environmentally controlled quarters (20–25 °C, relative humidity 55–65%, 12 h light/12 h dark cycle) for 5 days before experiment. The food and water were available all the time. The animal experiment designed in this study was approved by the ethical committee of Shanghai Jiao Tong University School of Medicine.