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Gambogic acid inhibits tumor cell adhesion by suppressing integrin β1 and membrane lipid rafts-associated integrin signaling pathway

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ABSTRACT

Cell adhesion plays an important role in the steps of cancer metastasis. Regulation of cell–cell (intercellular) and cell–matrix adhesion is a promising strategy for cancer progression. Gambogic acid is a xanthone derived from the resin of the Chinese plant *Garciania hanburyi*, with potent anti-metastasis activity on highly metastatic cells. The aim of this study was to investigate the function and mechanism of gambogic acid on tumor adhesion. We found that gambogic acid strongly inhibited the adhesion of human cancer cells to fibronectin. This inhibition was associated with the deformation of focal adhesion complex, which was mediated by suppressing the expression of integrin β1 and integrin signaling pathway. *In vitro*, cell lipid rafts clustering was inhibited following treatment of gambogic acid, which induced the suppression of integrin β1 and focal adhesion complex proteins colocalization within rafts. Moreover, gambogic acid significantly decreased cellular cholesterol content, whereas cholesterol replenishment lessened the inhibitory effect of gambogic acid on cell adhesion. Real-time PCR analysis showed that gambogic acid reduced mRNA levels of hydroxymethylglutaryl-CoA reductase and sterol regulatory element binding protein-2, while increased acetyl-CoA acetyltransferase-1/2. Taken together, these results demonstrate that gambogic acid inhibits cell adhesion via suppressing integrin β1 abundance and cholesterol content as well as the membrane lipid raft-associated integrin function, which provide new evidence for the anti-cancer activity of gambogic acid.

1. Introduction

Cancer cells are characterized by uncontrolled growth, invasion into surrounding tissues, and in many cases metastatic spread to distant sites, which is the primary cause of cancer-related mortality. Tumor metastasis occurs by a complex series of events, including cell adhesion, invasion, proliferation, and vessel formation [1]. The invasion of tumor cells into adjacent tissues is a crucial event in metastasis. Invasion of tumor cells involves multiple processes, which depend on specific cell-to-cell and cell-to-extracellular matrix (ECM) interactions [2]. It is indicated that anti-adhesion is an effective strategy for metastasis inhibition [3,4]. Therefore, anti-adhesion as one of the targets for treating tumor metastasis has been widely accepted and extensively investigated [5].

Cell adhesion is mediated directly by specific adhesion receptors, such as integrins, cadherins, selectins, and intercellular adhesion molecules [6]. Integrins and their downstream signaling play an important role in cell adhesion. Integrin is an α/β heterodimeric membrane protein that mediates cell adhesion to

Abbreviations: ACAT, acetyl-CoA acetyltransferase; BSA, bovine serum albumin; CTxB, cholera toxin B subunit; ECM, extracellular matrix; FAC, focal adhesion complex; FAK, focal adhesion kinase; FITC, fluorescein-5-isothiocyanate; FN, fibronectin; GA, gambogic acid; GC–MS, gas chromatography–mass spectrometry; HMG, hydroxy-methylglutaryl; LDL-r, low density lipoprotein receptor; MMP, matrix metalloproteinase; MBCD, methyl-β-cyclodextrin; PE, phycoerythrin; SREBP, sterol regulatory element binding protein; SR-BI, scavenger receptor class B type I.

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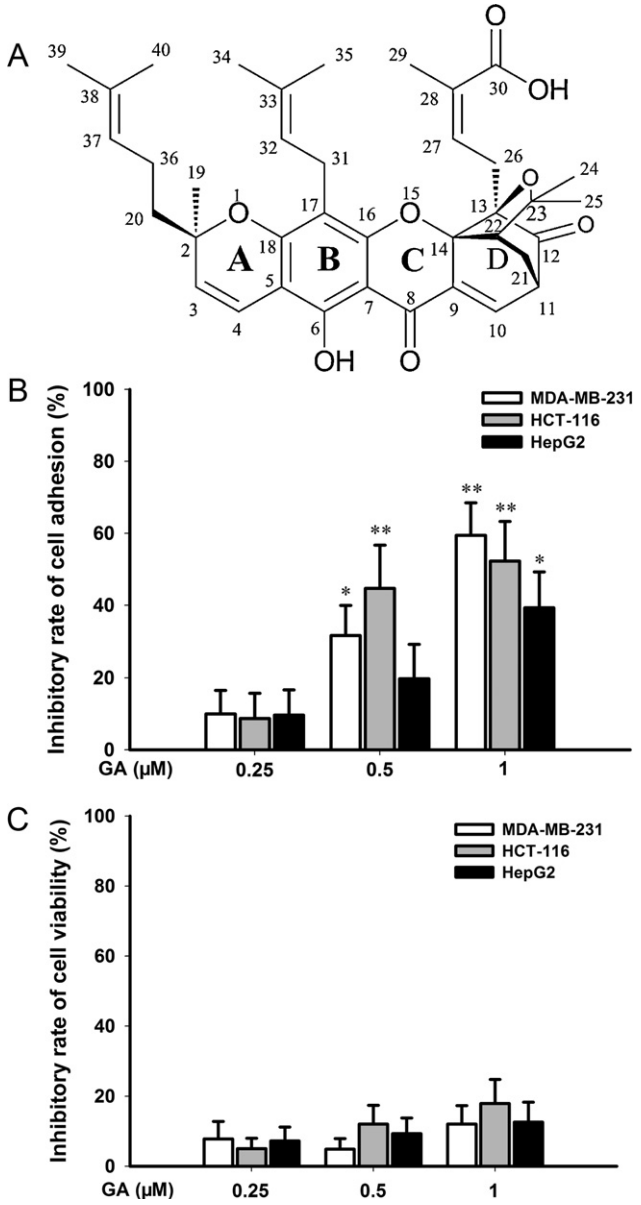

Fig. 1. GA inhibits cancer cell adhesion. (A) Chemical structure of GA. (B) Inhibitory effect of GA on adhesion of MDA-MB-231 cells, HCT-116 cells, and HepG2 cells to fibronectin. (C) Effect of GA on the viability of MDA-MB-231 cells, HCT-116 cells, and HepG2 cells. $^{*}P < 0.05$ or $^{**}P < 0.01$ represents significant difference from the control group.

components of the ECM. Integrin β1 subunit is crucial for adhesion to fibronectin (FN) [7], one important component of ECM. With interaction of ECM components, integrin molecules cluster at specific locations in the plasma membrane [8], which mediates transmembrane signal transduction via other signaling molecules recruited to focal adhesions [9], such as focal adhesion kinase (FAK), the kinase c-Src, paxillin, and vinculin [10]. It is also reported that integrin β1 is distributed in lipid rafts [11]. Lipid rafts (also known as detergent-resistant membrane or detergent-insoluble membrane) are at the cell surface membrane by being clustered with sphingomyelin and/or cholesterol [12]. Lipid rafts have been suggested to be important for modulation of signal transduction and cell adhesion [13]. The function of integrins, such as integrin α5β1, is positively regulated by lipid rafts when associated with fibronectin [14]. This regulatory effect of lipid rafts is also found on integrin α4β1 [15].

Gambogic acid (GA, Fig. 1A) is the major active ingredient of gamboges, a brownish to orange resin from the Chinese plant, *Garcinia hanburyi*. Previous studies demonstrated the molecular mechanism of apoptosis activation effect of GA by binding to the transferrin receptor [16] and suppressing nuclear factor-kappa B signaling pathway [17], as well as the anti-angiogenesis effect by suppressing vascular endothelial growth factor receptor 2 signaling [18]. Our work indicated the potent anti-metastasis activity of GA, which mainly attributed to its inhibition of matrix metalloproteinase (MMP)-2 and MMP-9 [19,20]. GA also inhibits integrin α4-mediated adhesion of B16-F10 cells [21]. Since cell adhesion is a key step in the metastatic cascade of cancers, it is essential to investigate the effect of GA on cancer cell adhesion and the mechanism involved in. These findings provide new evidence of the anti-metastatic activity of GA.

2. Materials and methods

2.1. Materials

GA (99% purity) was supplied by Dr. Qidong You's laboratory (China Pharmaceutical University, China), which was isolated from the resin of *Garciania hanburyi* and purified according to the established methods [22]. GA was prepared as described previously [19]. Fibronectin was from BD Biosciences (Bedford, MA, USA). Antibodies to integrin β1 (8A2) (sc-73645), FAK (H-1) (sc-1688), p-FAK (Tyr397)-R (sc-11765-R), paxillin (H-114) (sc-5574), vinculin (H-300) (sc-5573), c-Src (N-16) (sc-19), p-c-Src (9A6) (sc-81521), β-actin (9) (sc-130301), isotype-matched immunoglobulin G (IgG) (ICO-97) (sc-66186), and Protein A/G Plus-Agarose were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The cholesterol standard preparation (purity >99%), methyl-β-cyclodextrin (MBCD), bovine serum albumin (BSA), MTT, paraformaldehyde, Triton X-100, Tris, NaCl, EDTA, NP-40, PMSF, NaF, SDS, DTT, and fluorescein-5-isothiocyanate (FITC)-conjugated cholera toxin B subunit (CTxB) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Cell culture

MDA-MB-231 (a human breast carcinoma cell line), HCT-116 (a human colon carcinoma cell line), and HepG2 (a human hepatocarcinoma cell line) were purchased from the Cell Bank of the Shanghai Institute of Cell Biology (Shanghai, China). MDA-MB-231 cells, HCT-116 cells, and HepG2 cells were cultured in Leibovitz's L15, McCoy's 5A, and RPMI-1640 medium (Gibco, Invitrogen, Carlsbad, CA, USA), respectively, containing 10% fetal bovine serum (Gibco, Invitrogen, Carlsbad, CA, USA), 100 U/mL penicillin (Beyotime, Nantong, China), and 100 μg/mL streptomycin (Beyotime, Nantong, China). The cells were maintained in a humidified atmosphere of 95% air/5% CO_2 at 37 °C.

2.3. Cell adhesion assay

Cell adhesion assay was performed as described [21] with modifications. Ninety-six-well plates (BD Biosciences, Bedford, MA, USA) were coated with fibronectin (5 μg/mL) at 4 °C for overnight and then blocked in BSA (1%) for 1 h. Serum-starved cells were exposed to GA (0.25, 0.5, or 1 μM) for 24 h before seeding. Target cells were harvested and suspended in fetal bovine serum-free medium. Cells (2×10^5 mL^{-1}) were seeded to fibronectin-coated plates and then incubated for 1 h at 37 °C. Non-adherent cells were removed by gentle washing with PBS. Then, colorimetric MTT assay was employed to analyze the adhesion ability of cells.

2.4. Cell viability assay

Cells were seeded onto Falcon 96-well plates (BD Biosciences, Bedford, MA, USA) for 24 h. Then cells were exposed to GA (0.25,