Life Sciences 84 (2009) 915–922

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Sennoside B inhibits PDGF receptor signaling and cell proliferation induced by PDGF-BB in human osteosarcoma cells

Yen-Chun Chen [a,b,*], Chia-Ni Chang [a], Hui-Chun Hsu [a], Shu-Jiau Chiou [a], Lain-Tze Lee [a], Tzong-Hsiung Hseu [b]

[a] Biomedical Engineering Research Laboratories, Industrial Technology Research Institute, Hsinchu, Taiwan
[b] Institute of Biotechnology, National Tsing Hua University, Hsinchu, Taiwan

ARTICLE INFO

Article history:
Received 19 December 2008
Accepted 4 April 2009

Keywords:
Sennoside B
Osteosarcoma
Signaling pathway
Platelet-derived growth factor (PDGF)
Platelet-derived growth factor receptor (PDGFR)

ABSTRACT

Aims: To address the possibility that sennoside B inhibition of cell proliferation is mediated via interference with platelet-derived growth factor (PDGF) signaling.
Main methods: Human osteosarcoma MG63 cells were treated with PDGF in the presence or absence of sennoside B. Activation of the PDGF signaling pathway was monitored using western immunoblotting with specific antibodies against the PDGF receptor, phosphotyrosine and components of the downstream signaling cascade. Activation of cell metabolism and proliferation was assessed by chromogenic reduction of MTT.
Key findings: Sennoside B was found to inhibit PDGF-BB-induced phosphorylation of the PDGF receptor (PDGFR) in human MG63 osteosarcoma cells. Downstream signaling was also affected; pre-incubation of PDGF-BB with sennoside B inhibited the phosphorylation of pathway components including Ak strain transforming protein (AKT), signal transducer and activator of transcription 5 (STAT-5) and extracellular signal-regulated kinase 1/2 (ERK1/2). Further, we found that sennoside B can bind directly to the extracellular domains of both PDGF-BB and the PDGF-β receptor (PDGFR-β). The effect was specific for sennoside B; other similar compounds including aloe-emodin, rhein and the *meso* isomer (sennoside A) failed to inhibit PDGFR activation or downstream signaling. Sennoside B also inhibited PDGF-BB stimulation of MG63 cell proliferation.
Significance: These results indicate that sennoside B can inhibit PDGF-stimulated cell proliferation by binding to PDGF-BB and its receptor and by down-regulating the PDGFR-β signaling pathway. Sennoside B is therefore of potential utility in the treatment of proliferative diseases in which PDGF signaling plays a central role.

Introduction

Sennosides have been used as natural, safe laxatives in traditional and modern systems of medicine. In addition, aloe-emodin and rhein, degradation products of sennosides, have been reported to inhibit tumor cell growth (Cardenas et al. 2006; Huang et al. 2007). Doxorubicin, a structurally related anthraquinone glycoside, is an important anti-neoplastic drug (Huang et al. 2007), while emodin has been reported to induce apoptotic cell death in proliferating cells (Pecere et al. 2000; Huang et al. 2007). In view of the planar structure of these molecules, it has been suggested that anthraquinones may be able to intercalate into DNA and that this could underlie their cytoxicity.

Sennosides and related molecules may also exert their effects by interfering with the activation of cell-surface receptors. Emodin preferentially suppresses the phosphorylation of the human epidermal growth factor receptor 2 (HER-2/neu) in breast cancer cells (Zhang et al. 1995, 1998). Tyrosine kinase phosphorylation induced by epidermal growth factor (EGF) was also suppressed (Huang et al. 2005). Furthermore, it has been suggested that the anti-angiogenic activity of emodin may be due to inhibition of the phosphorylation of the vascular endothelial growth factor receptor 2 (VEGFR2) and of downstream molecules (Kaneshiro et al. 2006; Srinivas et al. 2007; Lu et al. 2008).

Platelet-derived growth factors (PDGFs) and their receptors have been implicated in the pathogenesis of a number of tumor types and play an important role in angiogenesis (Board and Jayson 2005; Pietras et al. 2003; Alvarez et al. 2006). Extensive experimental data highlight the potential therapeutic advantage of targeting the platelet-derived growth factor receptor (PDGFR). In humans, the PDGF signaling network consists of four ligands, PDGFs A through D. These factors interact with two receptors, PDGFR-α and PDGFR-β. Ligand-bound receptors undergo autophosphorylation, resulting in the activation of

* Corresponding author. Biomedical Engineering Research Laboratories, Industrial Technology Research Institute, Hsinchu, Taiwan. Tel.: +886 3 5732561; fax: +886 3 5732359.
E-mail address: YenChunChen@itri.org.tw (Y.-C. Chen).

0024-3205/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2009.04.003

multiple intracellular signaling cascades. Regarding PDGF, the best characterized intracellular signaling cascade comprises mitogen-activated protein kinase kinase/extracellular regulated kinase (MEK/ERK), phosphatidylinositol 3-kinase/Ak strain transforming protein (PI3K/AKT) and signal transducer and activator of transcription 5 (STAT-5) (Sandy et al. 1998; Graves et al. 1984; Thomas et al. 1997; Paukku et al. 2000; Valgeirsdottir et al. 1998; Zhang et al. 2007). Because this pathway has been widely implicated in the regulation of tumor growth, agents interfering with PDGF-BB signaling have great potential for the development of new anti-neoplastic agents.

MG63 osteosarcoma cells present a functional PDGFR but do not secrete PDGF-like mitogens (Graves et al. 1984); these cells have been used for many years in osteoblast research (Sandy et al. 1998). In the present study, we used MG63 cells to investigate the effects of sennoside B on PDGF-BB-induced signaling. Our results indicate that sennoside B specifically binds to PDGF-BB and PDGFR and can inhibit PDGFR activation and downstream signaling induced by PDGF-BB. In a cell-based functional study, we also found that sennoside B can inhibit MG63 proliferation induced by PDGF-BB. Sennoside B may have significant potential for anti-cancer therapy.

Materials and methods

Materials

Sennoside A, Sennoside B, aloe-emodin and rhein were obtained from Sigma Chemical Co (St. Louis, MO, USA). These compounds were dissolved in DMSO (Sigma Chemical Co) and further diluted in Dulbecco's Modified Eagle's Medium (DMEM) without fetal bovine serum (FBS; Biological Industries). Penicillin, DMEM, and streptomycin were obtained from GIBCO™/Invitrogen Life Technologies (CA, USA). Bovine serum albumin (BSA) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were from Sigma Chemical Co. Antibodies raised against PDGFR-β and anti-phosphotyrosine antibody (PY99) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phosphotyrosine monoclonal antibody (4G10) was from Upstate Biotech (Lake Placid, NY, USA). Recombinant human PDGF-BB, the PDGFR-β/Fc chimera, EGF and antibodies raised against native PDGF-BB, PDGFR-β and ERK1/2, AKT, STAT5 were from R&D Systems (MN, USA). Antibodies raised against phospho-ERK1/2 (Thr202/Tyr204), phospho-AKT (Ser473, Thr308), phospho-STAT5 (Tyr694), Akt and horseradish peroxidase (HRP)-linked anti-goat and anti-mouse IgG antibodies, as well as the cell lysis buffer, were from Cell Signaling Technology (Danvers, MA, USA). Antibodies raised against EGFR were from LabVision (Fremont CA, USA). Pre-stained protein markers were from Fermentas Life Sciences (Glen Burnie, MD, USA). SuperSignal® West Pico HRP chemiluminescence substrate, the Micro BCA™ Protein Assay Reagent protein assay kit and Western Blot Stripping Buffer were from Pierce (Rockford, IL, USA). Nitrocellulose membranes (Hybond-C Extra) were from Amersham Biosciences (Sunnyvale, CA, USA), and PVDF membranes were from Millipore (Billerica, MA, USA).

Osteosarcoma cell culture

Human osteoblast-like cells MG63 (BCRC-60279) and U-2OS (BCRC-60187) were obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan). Saos-2 cells (HTB-85) were obtained from The American Type Culture Collection (VA, USA) and grown in DMEM supplemented with 5–10% FBS, penicillin (100 units/ml) and streptomycin (100 μg/ml). Unless otherwise indicated, cells reaching 80–90% confluence were starved and synchronized in DMEM without FBS at 37 °C for 24 h before further analysis. For western blot analysis, osteosarcoma cells were seeded into 6-well plates (Orange Scientific, Belgium) such that each well contained 1 ml of a 5×10^5 cells/ml suspension (as assessed by hemocytometry). Cells were then cultured for 24 h, and then incubated in serum-free medium overnight before preparation of cell lysates.

Cell lysate preparation and western blot analysis

Osteosarcoma cells were cultured with PDGF-BB and sennosides for the times and at the different concentrations indicated in the figure legends. Lysates were prepared by the addition of cell lysis buffer and were clarified by centrifugation (14,000 g, 10 min, 4 °C). Lysate protein contents were quantified using the Pierce protein assay kit according to the manufacturer's instructions, using bovine serum albumin (BSA) as the reference standard. Proteins were separated by electrophoresis on SDS-polyacrylamide gels, electroblotted onto PVDF or NC membranes and probed using primary anti-phosphotyrosine, anti-p-ERK, anti-p-AKT, and anti-p-STAT5 antibodies. Immunoblots were developed using enhanced chemiluminescence (ECL) with HRP-labeled secondary antibodies and the SuperSignal® HRP substrate. The membranes were stripped (Western Blot Stripping Buffer), washed and reprobed with anti-PDGFR-β, anti-ERK1/2, anti-AKT or anti-STAT-5 antibodies and developed as described above.

Dot-binding assay

Aliquots (2.5 μl) of vehicle (DMSO) and different concentrations of sennoside, PDGF-BB or PDGFR-β/Fc chimera in vehicle were spotted onto gridded PVDF membranes and air-dried. Membranes were blocked with BSA (5% in PBS) for 0.5 h. After washing in PBS, membranes were incubated with PDGF-BB or the PDGFR-β/Fc chimera (1 μg/ml) for 1 h at room temperature in PBS. Following a brief wash, the membrane was incubated with anti-PDGF-BB or PDGFR-β extracellular domain antibody (2 μg/ml in PBS containing 1% BSA) for 1 h at room temperature. After another brief wash, the membrane was incubated with HRP-conjugated secondary antibody and developed by ECL.

Cell proliferation assays

Activation of cell metabolism and proliferation as reflected by NAD-dependent dehydrogenase activity was determined via chromogenic reduction of MTT. Osteosarcoma cells in a 96-well plates were allowed to reach 50% confluence (5×10^3 cells/well), starved for 24 h and incubated with different concentrations of sennoside B or vehicle (final concentration 0.5% DMSO) in the absence or presence of PDGF-BB (20 ng/ml) in quintuplicate. After 24 and 48 h, cells were incubated with 0.5 mg/ml MTT for 2 h at 37 °C. Formazan crystals resulting from MTT reduction were dissolved by adding 200 μl DMSO and gently

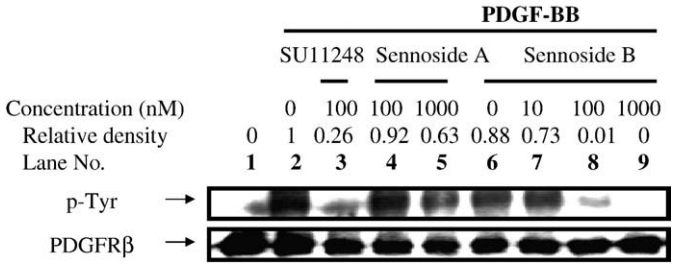

Fig. 1. PDGFR phosphorylation in MG63 cells treated with PDGF-BB and sennosides A and B. MG63 cells were treated with medium containing PDGF-BB (20 ng/ml), with vehicle or the indicated concentrations of sennoside A or B for 5 min. Multiple kinase inhibitor SU11248 was included as a positive control. Cell lysates were collected, and protein tyrosine phosphorylation (p-Tyr) and total PDGFR-β were determined by western blotting. The 180-kDa phosphotyrosine band (detected by PY99) in the upper panel was identified as tyrosine-phosphorylated PDGFR. Relative band densities were quantified by scanning densitometry and normalized to the relative value of total PDGFR-β. The data shown are representative of three or more independent experiments.