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Diosgenin induces death receptor-5 through activation of p38 pathway and promotes TRAIL-induced apoptosis in colon cancer cells

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ABSTRACT

Previously, we demonstrated that diosgenin induced apoptosis in colorectal cancer cell lines HCT-116 and HT-29. HT-29 cells have been reported to be one of the most resistant colorectal cancer cell lines to TRAIL-induced apoptosis. In this study, we investigated the effect of diosgenin on TRAIL-induced apoptosis in HT-29 cells. We showed that diosgenin sensitizes HT-29 cells to TRAIL-induced apoptosis. Mechanisms underlying this sensitization mainly involved diosgenin-induced p38 MAPK pathway activation and subsequent DR5 overexpression. Furthermore, we showed that diosgenin alone, TRAIL alone or combination treatment increased COX-2 expression and that the use of a COX-2 inhibitor further increased apoptosis induction.

1. Introduction

Selectively inducing apoptosis in cancer cells has been increasingly recognized as a promising therapeutic approach for many cancers, including colorectal cancer. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL or Apo2 ligand) is a member of the tumor necrosis factor (TNF) cytokine family [1]. TRAIL is able to selectively induce apoptosis in tumor cells *in vitro* [1,2] and, most importantly *in vivo* [3,4] with no significant untoward effect on normal cells.

Apoptosis induced by TRAIL is initiated by its binding to death receptors (TRAIL-R1 or DR4 and TRAIL-R2 or DR5) followed by formation of the death-inducing signalling complex (DISC) upon recruitment of specific cytoplasmic proteins, Fas-associated death domain (FADD) and caspase-8 or -10 [5]. Two other membrane bound receptors, DcR1 and DcR2, lack the cytoplasmic region and have truncated intracellular death domains, respectively, and cannot induce apoptosis.

Although most cancer cells express functional TRAIL receptors DR4 and DR5, resistance to TRAIL's cytotoxic effects is common. Many molecular mechanisms may account for these cancer cells' resistance to TRAIL. Determinants of TRAIL sensitivity are reported to include the expression levels of death receptors (DR), FLICE inhibitor protein (FLIP) and Bcl-XL, but such relationships are not observed in all cell lines [6,7]. TRAIL sensitivity in tumor cell lines can be modulated by p53, MAP kinase pathways, c-Myc, PI3K/AKT pathway and NF-κB signalling [8,9]. Inhibition of PI3K/AKT activation or inhibition of NF-κB signalling by various agents has been shown to enhance TRAIL cytotoxicity in numerous cell models [10–12].

Thus, agents that can up-regulate TRAIL receptors or inhibit survival pathways, such as chemotherapeutic agents or natural products, have the potential to enhance the apoptotic effects of TRAIL [13–18].

Abbreviations: TRAIL, the tumor necrosis factor (TNF) α-related apoptosis-inducing ligand; DR, death receptor; MAPK, mitogen activated protein kinase; COX-2, cyclooxygenase-2.

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Diosgenin, a steroid saponin present in fenugreek (*Trigonella foenum graecum*) and other plants, is known for its antitumor effects on cancer cells [19]. Diosgenin inhibits the growth of cancer cell lines through cell cycle arrest, inhibition of PI3K/Akt and NF-κB survival pathways and induction of apoptosis [20–22]. In our previous study, we demonstrated that diosgenin induced apoptosis in colorectal cancer cell lines HCT-116 and HT-29 [23]. After diosgenin treatment, we observed apoptosis hallmarks in both cell lines but HT-29 cells were more resistant with delayed apoptosis. On the other hand, HT-29 cells have also been reported to be one of the most resistant colorectal cancer cell lines to TRAIL-induced apoptosis [24]. Therefore, a combinatorial strategy using diosgenin seemed to be potentially promising for overcoming TRAIL resistance in colorectal cancer cells.

Cyclooxygenase-2 (COX-2) expression is usually involved in colorectal cancer pathogenesis [25,26]. Tang et al. [27] showed that COX-2 overexpression inhibited DR5 expression and attenuated TRAIL-induced apoptosis in human colon cancer cells and this inhibition was restored by the COX-2 inhibitor sulindac. The causal relationship between arachidonic acid metabolism and apoptosis induced by TRAIL must be probed to establish whether it is a well-founded target in colorectal cancer treatment.

In the current study, we investigated the effect of diosgenin on TRAIL-induced apoptosis in human colon cancer cells HT-29. We showed for the first time that diosgenin sensitizes HT-29 colon cancer cells to TRAIL-induced apoptosis. Mechanisms underlying this sensitization mainly involved diosgenin-induced p38 MAPK signalling pathway activation and subsequent DR5 overexpression in HT-29 cells. Furthermore, diosgenin also activated apoptotic effectors and inhibited survival pathways thus amplifying TRAIL-induced apoptosis. On the other hand, we showed that diosgenin alone, TRAIL alone or a combination of both increased COX-2 expression and that the use of a COX-2 inhibitor further increased apoptosis induction. Taken together, these results suggest that the combination of diosgenin with TRAIL may be a promising candidate for treatment of TRAIL-resistant colon cancer cells.

2. Materials and methods

2.1. Cell lines, cell culture, treatment and light microscopy

The HT-29 cell line was purchased from American Culture Type Collection (LGC Standards, Middlesex, United Kingdom). Cells were seeded at 5×10^6 cells in 75 cm^2 tissue culture flasks, grown in DMEM medium (Gibco BRL, Cergy-Pontoise, France) supplemented with 5% fetal calf serum (FCS) (Gibco BRL), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco BRL). The HCT-116 cell line was purchased from American Culture Type Collection (LGC Standards). Cells were seeded at 3×10^6 cells in 75 cm^2 tissue culture flasks, grown in MEM medium (Gibco BRL) supplemented with 5% fetal calf serum (FCS) (Gibco BRL), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco BRL). The HEL human erythroleukemia cell line was kindly provided by Pr JP. Cartron (INSERM U76, Paris, France). Cells were seeded at 10^5 cells/ml in 75 cm^2 tissue culture flasks and grown in RPMI 1640 medium (Gibco BRL) as previously described [28,29]. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 °C. Cells were grown for 36 h in culture medium prior to exposure or not to 20 or 40 μM diosgenin (Sigma Aldrich, Saint Quentin Fallavier, France) 6 h before adding or not 20 ng/mL TRAIL (R&D System, Lille, France). A stock solution of 10^{-2} M diosgenin was prepared in ethanol; a 20 μg/mL stock solution of TRAIL was prepared in phosphate-buffered saline (PBS) – 0.1% BSA, stock solutions were then diluted in culture medium to give the appropriate final concentration. The same amount of vehicle was added to control cells. Cell viability was determined by the trypan blue dye exclusion method. For light microscopy, after treatment, cultured cells were examined under phase-contrast microscopy (magnification 400×), and pictures were taken with an image acquisition system (Nikon, Champigny sur Marne, France).

When the pharmacological inhibitor of COX-2, celecoxib, was used, cells were treated or not simultaneously with 10 μM celecoxib and 20 or 40 μM diosgenin for 6 h before adding or not 20 ng/mL TRAIL for 24 h. For p38 inhibition, cells were pretreated with 10 μM SB203580 (Calbiochem, La Jolla, CA, USA) for 2 h and then treated or not with 40 μM diosgenin for 6 h before adding or not 20 ng/mL TRAIL for 24 h.

2.2. Apoptosis quantification: DNA fragmentation

HT-29 cells were seeded at 5×10^6 cells in 75 cm^2 tissue culture flasks and then treated as described above. Apoptosis was quantified on floating and adherent cells using "cell death" enzyme-linked immunosorbent assay (ELISA) (Cell Death Detection ELISA^{PLUS}, Roche Diagnostics). Cytosol extracts were obtained according to the manufacturer's protocol and apoptosis was measured as previously described [20].

2.3. Protein expression analysis

After treatment, cells were washed and lysed in RIPA lysis buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 1% deoxycholate, 1% NP-40, 0.1% SDS, 20 μg/ml aprotinin) containing protease inhibitors (*Complete™ Mini*, Roche Diagnostics, Meylan, France). Western blot was performed as previously described [30]. Briefly, proteins (10–100 μg) were separated by electrophoresis on SDS–polyacrylamide gels, transferred to PVDF membranes (Amersham Pharmacia Biotech, Saclay, France) and probed with respective antibodies against poly(ADP-ribose) polymerase (PARP), procaspase-3, phospho-Akt, phospho-p38, DR4 and DR5 (Santa Cruz Biotechnology; TEBU, Le Perray en Yvelines, France), against caspase-8, caspase-9 and Bid (Cell Signalling Technology, Ozyme) and against COX-2 (Cayman Chemical). After incubation with secondary antibodies (Dako), blots were developed using enhanced chemiluminescence reagents (Amersham). Membranes were then reblotted with anti-β-actin monoclonal antibody (Sigma). Western blots were analyzed by densitometry (GBOX,