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Penta-acetyl geniposide-induced apoptosis involving transcription of NGF/p75 via MAPK-mediated AP-1 activation in C6 glioma cells

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Abstract

We have demonstrated the herbal derivative penta-acetyl geniposide ($(Ac)_5GP$) induces C6 glioma cell apoptosis through the critical sphingomyelinase (SMase)/nerve growth factor (NGF)/p75 and its downstream signals. It has been reported mitogen-activated protein kinase (MAPK) mediates NGF synthesis induced by SMase activation. In this study, ERK, p38 and JNK are shown to mediate $(Ac)_5GP$-induced glioma cell apoptosis and elevation of NGF and p75. Treatment of PD98059 (ERK-specific inhibitor), SB203580 (p38 MAPK inhibitor) and SP600125 (JNK inhibitor) decreases the elevation of NGF and p75 mRNA induced by $(Ac)_5GP$, indicating possible transcription regulation via MAPKs. The results of nuclear extract blotting and EMSA further confirm $(Ac)_5GP$ maximally increases AP-1 and NF-κB DNA binding at 6 h. Inhibition of ERK, p38 and JNK block the activation of AP-1 and NF-κB, suggesting these MAPKs are involved in $(Ac)_5GP$-induced transcription regulation. We thereby used RT-PCR to analyze cells treated with $(Ac)_5GP$, with or without AP-1 or NF-κB inhibitors. AP-1 inhibitor NDGA decreases NGF/p75 and expression of FasL and caspase 3 induced by $(Ac)_5GP$, suggesting the importance of AP-1 in mediating NGF/p75 and their downstream apoptotic signals. However, FasL and caspase 3 do not change with the NF-κB inhibitor PDTC; NF-κB might be linked to other cellular events. Overall, we demonstrate that MAPK mediates $(Ac)_5GP$-induced activation of AP-1, promoting the transcription of NGF/p75 and downstream apoptotic signals. These results further highlight the potential therapeutic effects of $(Ac)_5GP$ in chemoprevention or as an anti-tumor agent.

Keywords: Penta-acetyl geniposide ($(Ac)_5GP$); C6 glioma cells; NGF; p75; MAPK; ERK; p38; JNK; AP-1; NF-κB; Apoptosis

Abbreviations: $(Ac)_5GP$, penta-acetyl geniposide; SMase, sphingomyelinase; NGF, nerve growth factor; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated protein kinase; JNK, c-Jun NH_2-terminal kinase; RT-PCR, reverse transcription polymerase chain reaction; AP-1, activator protein-1; NF-κB, nuclear factor-κB; EMSA, electrophoretic mobility shift assay; NDGA, nordihydroguaiaretic acid; PDTC, pyrrolidone dithiocarbamate; PKCδ, protein kinase Cδ

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1. Introduction

Penta-acetyl geniposide [1-(β-D-2′,3′,4′,6′-tetra-acetyl-glucopyranosyloxy)-1,4*a*,5,7*a*-tetrahydro-7-(acetomethyl)-cyclopentapyran-4-carboxylic acid methyl ester; $(Ac)_5GP$] (Fig. 1), an herbal acetylated derivative of *Gardenia geniposide*, has been suggested as a potent chemopreventive agent which reduces DNA damage, activates detoxication enzymes and possesses anti-tumor effects through apoptosis and growth arrest (Peng et al., 2005a).

In a previous study, we demonstrated that $(Ac)_5GP$ transduced the apoptosis of C6 glioma cells through sphingomyelinase (SMase)/nerve growth factor (NGF)/p75 and their downstream signals (Peng et al., 2006). The activation or generation of ceramide and downstream NGF/p75 has been suggested critical in $(Ac)_5GP$-induced glioma cell apoptosis (Peng et al., 2004, 2005b). It has been reported that SMase mediates NGF synthesis via the activation of mitogen-activated protein kinase (MAPK) in cultured astrocytes (Galve-Roperh et al., 1997). Other investigations also suggest that MAPK mediates downstream SMase or ceramide (Raines et al., 1993).

MAPK is a serine-threonine kinase consisting of at least three subfamilies, namely extracellular signal-regulated protein kinase (ERK), c-Jun-N-terminal kinase (JNK) and p38 kinase, which mediates the regulation of cell proliferation, survival, differentiation and apoptosis (Johnson and Lapadat, 2002). ERK and JNK activate activator protein-1 (AP-1) thus mediate NO-induced apoptosis of cardiomyocytes (Taimor et al., 2001). JNK/AP-1 activation has also been shown to be involved in tumor cell apoptosis (Lauricella et al., 2006). In addition, JNK and p38 regulate AP-1 binding thus mediate the response to cell injuries (Chen et al., 2003; Tanos et al., 2005). However, p38 has been shown to suppress apoptosis via downstream nuclear factor-κB (NF-κB) activation (Craig et al., 2000).

Recently, it has been shown that ceramide-induced apoptotic cascades is mediated by activation and cooperation of JNK and p38 (Willaime-Morawek et al., 2003). As to liver cells and neurons, JNK and p38 are necessary for ceramide-induced apoptosis, respectively (Giuliano et al., 2006; Willaime et al., 2001). Ceramide treatment can induce apoptosis accompanied by JNK, NF-κB and AP-1 activation (Manna et al., 2000).

Since NGF promoter contains an AP-1 consensus sequence, some evidence indicates the relevance of AP-1 activation in NGF expression (Veenstra et al., 1998). In C6 glioma cells, there exists a correlation between increased AP-1 NGF binding and the induction of NGF transcription (Colangelo et al., 1996). Cotransfection with plasmids showed that both c-fos and c-jun are necessary to elevate NGF mRNA levels (Omae et al., 1994). In addition, NF-κB has been reported to be involved in neurotrophin expression (Friedman et al., 1996). Inflammatory cytokines interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and neurotoxicity-mediating peptide A beta are potent stimulators of microglial NGF synthesis involving the NF-κB-dependent mechanism (Heese et al., 1998b). However, little is known about neurotrophin receptor p75 transcription regulators.

In this study, we investigate whether MAPKs, including ERK, JNK and p38, are involved in $(Ac)_5GP$-transduced NGF/p75 expression, and the possible regulators for NGF and p75 transcription, mediating downstream apoptotic signals.

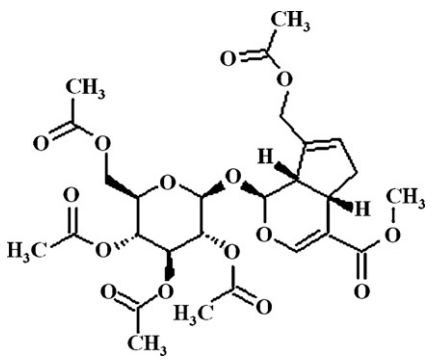

Fig. 1. Structure of $(Ac)_5GP$.

2. Materials and methods

2.1. Chemicals

Minimal essential media, fetal calf serum, penicillin, and streptomycin were purchased from Gibco BRL (Grand Island, NY, USA). SDS, bis-acrylamide, ammonium persulfate, TEMED, and nitrocellulose membrane came from Bio-Rad Laboratories (Hercules, CA, USA). Tris–HCl, anti-β-actin, anti-mouse IgG, and anti-rabbit IgG came from Sigma Chemical Co. (St. Louis, MO, USA). Inhibitors of nordihydroguaiaretic acid (NDGA), pyrrolidone dithiocarbamate (PDTC), PD98059, SB203580, and SP600125 were also purchased from Sigma Chemical Co. (St. Louis, MO, USA). The LightShift™ EMSA Optimization & Control Kit and Chemoluminescent Nucleic Acid Detection Modules are products of Pierce (Rorkford, IL, USA). Phospho-c-jun, and phospho-JNK antibody were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA). Phospho-p38 antibody was purchased from BD Transduction Laboratories (Lexington, KY, USA). Antibodies of ERK, phospho-ERK, JNK, p38, NGF, NGFR