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NF-κB-dependency and consequent regulation of IL-8 in echinomycin-induced apoptosis of HT-29 colon cancer cells

Ju Youn Park [a], Jae-Ho Chang [b], Keum Seok Bae [c,*], Kyoung Ho Lee [a], Sun Ju Choi [a], Joo Young Park [a], Yong Suk Ryang [d], Soo-Ki Kim [a,b,**]

[a] Department of Microbiology, Yonsei University Wonju College of Medicine, Wonju 220-701, Republic of Korea
[b] Institute of Lifelong Health, Institute of Basic Medical Science, Yonsei University Wonju College of Medicine, Wonju 220-701, Republic of Korea
[c] Department of General Surgery, Yonsei University Wonju College of Medicine, Wonju 220-701, Republic of Korea
[d] Department of Biomedical Laboratory Science, College of Health Science, Yonsei University, Wonju 220-710, Republic of Korea

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Abstract

The present study was to see whether echinomycin-induced apoptosis would be NF-κB-dependent and if so, whether echinomycin would activate or inhibit NF-κB as well as resultant chemokine IL-8 expression. In HT-29 cells echinomycin activated NF-κB in time-dependent manner. EMSA in the presence of antibodies specific for p50 and p65 subunits indicated that echinomycin-induces the translocation of p50–p65 heterodimeric subunits of NF-κB. Levels of IκB were detected at initial echinomycin treatment and thereafter decreased, faintly seen after a 6 h treatment. In contrast p-IκB levels were clearly detected throughout 6–24 h of echinomycin treatment, albeit initially fainted. To clarify the role of NF-κB on IL-8 expression in echinomycin-mediated apoptosis of HT-29 cells, ELISA plus RT-PCR clearly showed that IL-8 production is inducible by echinomycin treatment. Using a specific inhibitor, IL-8 regulation at echinomycin treatment in HT-29 cells occurred via both caspase-3 and NF-κB-dependent signal pathway. To confirm whether two different pathways (NF-κB and caspase) would be coupled, only NF-κB inhibitor (PDTC) and caspase-3 specific inhibitor (Z-DEVD-FMK) together significantly attenuated echinomycin-initiated apoptosis of HT-29 cells, pretreatment of HT-29 cells with PDTC rarely affected echinomycin-induced caspase-3 activation. So echinomycin-induced apoptosis in HT-29 cells occurs via NF-κB activation independent of caspase-3 activation modulating the resultant-linked key chemokine IL-8 expression and echinomycin-induced apoptosis is NF-κB-dependant and directly related to NF-κB activation, consequently regulating IL-8 expression.

Keywords: Echinomycin; NF-κB; IL-8; HT-29 cells

1. Introduction

DNA is a favored target for cancer chemotherapy (July et al., 2004; Chen and Horwitz, 2002). Of DNA damaging anticancer agents, double-strand break (DSB) inducers such as topoisomerase inhibitors (Jacob et al., 2005), anthracycline (Capranico et al., 1987) and DNA intercalating agents (e.g. echinomycin) have been used preclinically or clinically for treatment of a variety of solid cancers (Ryu et al., 2000). These compounds directly attack random or specified site of cancer cell DNA and also trigger a wide array of intracellular signaling pathways. Both direct and delicate, a complex network of genotoxic signals synergistically induce efficient apoptosis of targeted cancer cells (Janssens and Tschopp, 2006; Shin et al., 2006; D'Agostini et al., 2005). While the apoptotic signal path of DSB inducer has been well documented, how DNA intercalating agents initiate or elicit apoptotic signaling is unknown.

Previous reports have presented clear evidence regarding the existence of mitochondrial dependent, MAP kinase pathway in echinomycin, the prototypic DNA bis-intercalator treated HT-29 colon cancer cells. This discovery might confer overall insight

* Corresponding author. Tel.: +82 33 741 1306; fax: +82 33 742 5034.
** Corresponding author. Tel.: +82 33 741 0323; fax: +82 33 742 5034.
E-mail addresses: bksgs@yonsei.ac.kr (K.S. Bae), kim6@yonsei.ac.kr (S.-K. Kim).

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into unraveling the exact specified intracellular signal path taken by each DNA intercalator or minor groove binder (MGB) because these compounds would be good candidates because of their unique cytotoxic profiles against chemoresistant or intractable types of cancer (Geroni et al., 2002; Baraldi et al., 2001).

These signal pathways in echinomycin-induced apoptosis are mainly at the cytoplasmic level. The apoptotic signal might converge on the nucleus, consequently modulating nuclear factor κB (NF-κB) transcription factor. Emerging data suggest that a DSB inducer would directly trigger an NF-κB pathway, thereby leading to apoptosis via activating or suppressing NF-κB (Habraken and Piette, 2006). Despite partial knowledge of DSB-initiated NF-κB, the relaying molecular events between cytoplasmic signaling a path and an intra nuclear pathway of DNA intercalating agent in initiating apoptosis remains unknown.

Transcription factor − the inhibitory proteins NF-κB and IκB − can be phosphorylated, ubiquitinated and degraded by proteasomes, which results in upregulation of NF-κB mediated gene expression of inflammation and cancer. NF-κB is involved in inflammatory diseases, oncogenesis and apoptotic processes induced by cytokine, chemokine and antitumor drugs (Mayo and Baldwin, 2000; Barkett and Gilmore, 1999). Cytokines such as tumor necrosis factor (TNF)-α, interferon-β, interleukin-1 (IL-1), IL-6, and IL-8 and the adhesion molecules endothelial-leukocyte adhesion molecule (ELAM)-1, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 are products of some of the genes regulated by NF-κB-dependent mechanisms (Baeuerle and Baltimore, 1996). In an intestinal epithelial cell such as HT-29, NF-κB can be a central regulator of chemokine gene expression (Sougioultzis et al., 2006; Jijon et al., 2005). This chemokine may play some role in survival or death of cancer cells.

Of the chemokines, interleukin (IL)-8 is closely linked with NF-κB pathway in HT-29 cells (Mormina et al., 2006). IL-8 expression is closely linked with NF-κB or caspase pathway involved cell death; for instance, IL-8 gene induction is well documented in paclitaxel or its analogs induce cancer cell death via JNK and NF-κB pathway (Watson et al., 1998; Lee et al., 1998). In glioma cells, TRAIL can induce apoptosis via caspase dependent pathways, therefore signaling upstream of caspase-3 is indispensable for IL-8 release (Choi et al., 2001). HDAC inhibitor, apicidin-induced apoptosis is NF-κB-dependent leading to increased IL-8 expression (Kim et al., 2006). We hypothesized that echinomycin might affect IL-8 expression throughout the apoptotic process and the connective link between NF-κB. IL-8 expression in echinomycin-mediated apoptosis of HT-29 cells is also unknown.

We investigated whether echinomycin-induced apoptosis is NF-κB-dependent, and whether echinomycin could activate or inhibit NF-κB as well as the resultant chemokine expression of IL-8.

2. Materials and methods

2.1. Cell culture and chemotherapeutics

Human colon cancer cell line HT-29 was purchased from American Type Culture Collection (ATCC) (Rockville, MD, USA). HT-29 cells were cultured in RMPI1640 (Gibco BRL, Hercules, CA, USA) and supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 U/ml) in an atmosphere of 5% CO_2 in air at 37 °C. Echinomycin (Sigma, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO, USA) and added to the culture medium at the indicated concentration. The concentration of DMSO in the medium was <0.1% (v/v). Cells were incubated 37 °C for the indicated times and harvested.

2.2. Preparation of cell extracts

Cells from a dish were harvested, pelleted and washed in phosphate-buffered-saline (PBS) and they were resuspended in an equal volume of lysis buffer (100 mM Tris, 150 mM NaCl, 10% glycerol, 0.6% Triton-X 100, 5 mM EDTA, 1 mM sodium orthovanadate, 10 mM sodium fluoride and 2 mM phenylmethylsulfonyl fluoride (PMSF)). The cells were incubated for 60 min on ice and centrifuged at 14,000 rpm for 30 min at 4 °C. The soluble fraction was transferred to a new tube and the preparation was stored at −70 °C.

2.3. Western blot analysis

HT-29 cells were seeded in 35-mm plastic dishes (3×10^5 cells per dish) and incubated with echinomycin for different time periods. Cells were lysed in the lysis buffer (50 mM Tris−HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 1 μg/ml aprotinin, 10 μg/ml leupeptin, 1 μg/ml pepstatin A, and 1 mM sodium orthovanadate). After centrifugation at 15,000 rpm at 4 °C for 30 min supernatant was collected, 20 μg of lysates from each sample was run on 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel and electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes. PVDF membranes were rinsed in TBST (10 mM Tris−HCl (pH 7.4), 0.9% NaCl, 0.05% Tween 20 and 1 mM EDTA) and blocked in blocking buffer (TBST containing 5% bovine serum albumin) overnight at 4 °C. PVDF membranes were incubated with primary antibodies overnight at 4 °C, washed and incubated with goat anti-rabbit IgG conjugated with horseradish peroxidase (HRP) or goat anti-mouse IgG conjugated with HRP for 1 h at room temperature. The membrane was developed with electro-generated chemiluminescent (ECL) substrate (Amersham Life Sciences, Arlington Heights, IL, USA) and exposed to Biomax MS autoradiography X-ray film (Kodak, Rochester, NY, USA).

2.4. Transient transfection and luciferase reporter gene assay

The pGL3-NF-κB promoter, pCMV-IκBαM (Clontech, CA, USA) and pGL3-IL-8 promoter constructs were transfected into HT-29 cells by LipofectAmine 2000 (Invitrogen, California, CA, USA). The pGL3- IL-8 promoter was kindly provided by Dr Sohn M.H (University of Yonsei, Republic of