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Bcl-2 decreases cell proliferation and promotes accumulation of cells in S phase without affecting the rate of apoptosis in human ovarian carcinoma cells

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Abstract

Objectives. The Bcl-2 protein is an important regulator of the apoptotic cascade and promotes cell survival. Bcl-2 can also delay entry into the cell cycle from quiescence. In the present study, we used two isogenic human ovarian carcinoma cell lines, which expressed differential levels of Bcl-2 proteins, to demonstrate that Bcl-2 may regulate the growth rates of adenocarcinoma cells.

Methods. The growth rates of two isogenic ovarian cancer cell lines were determined by XTT assays and flow cytometry combined with PI staining. Bcl-2-overexpressing SKOV3 cells were modified to express a doxycycline-inducible anti-Bcl-2 single-chain antibody and the effects of Bcl-2 protein inhibition on cell proliferation and apoptosis were assessed.

Results. We demonstrate that Bcl-2 promotes the accumulation of proliferating carcinoma cells in S phase. The Bcl-2-overexpressing SKOV3 cell line proliferates markedly faster and shows delayed progression to G_2M phase compared to its low Bcl-2-expressing counterpart SKOV3.ip1 cell line. Single-chain antibody-mediated inhibition of Bcl-2 in SKOV3 cells was associated with increased growth rates and more rapid cell cycle progression. Treatment with cisplatin resulted in more cells accumulating in S phase in Bcl-2-overexpressing SKOV3 cells, while the inhibition of Bcl-2 abolished delayed entry into G_2M phase without affecting cisplatin-induced apoptosis.

Conclusions. Our results suggest that, in ovarian cancer cells, Bcl-2 delays cell cycle progression by promoting accumulation of cells in S phase without affecting the rate of apoptosis. Thus, in addition to its known role at the G0/G1 checkpoint, we demonstrate for the first time that Bcl-2 also regulates the S phase.

Keywords: Cisplatin; Apoptosis; Bcl-2; Ovarian cancer; Single-chain antibodies; Cell cycle

Introduction

Apoptosis plays a critical role in cellular homeostasis and prevents the development of tumor cells. The Bcl-2 family of proteins plays an important role in the regulation of apoptosis induced by a variety of stimuli [1]. The susceptibility of cells to a death signal appears to be determined, at least in part, by the ratio between pro- and anti-apoptotic proteins, which can heterodimerize and titrate one another's function [2–4]. In many instances, the expression of Bcl-2 protects from apoptosis induced by cytotoxic agents. The anti-apoptotic Bcl-2 protein resides on the cytoplasmic face of the mitochondrial outer membrane, endoplasmic reticulum (ER), and nuclear envelope, and may affect the transport of small molecules in these organelles through ion-channel activity [5]. Bcl-2, as a regulator of apoptosis, is participating to mitochondrial permeability transition through its ion-channel activity and inhibits the release of both cytochrome *c* and apoptosis-inducing factor (AIF) from mitochondria, thereby promoting cell survival [5].

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In addition to its role in regulating apoptosis, studies have revealed that Bcl-2 may retard cell cycle progression. Using cultured fibroblasts, it was shown that Bcl-2 expression delays serum-induced G_1/S phase transition [6] and accelerates withdrawal from the cycle [7]. In IL-3-dependent hemopoietic cells arrested in G_0, the expression of Bcl-2 retarded re-entry in the cell cycle of IL-3-stimulated cells [8,9]. More recently, investigation into the mechanisms of Bcl-2 effect on cell cycle has shown that Bcl-2 retards cell cycle entry by increasing p27 and p130 levels and by negatively regulating E2F1 [10,11]. Myc-induced S phase entry can also be blocked by Bcl-2 expression [12]. Interestingly, the ability of Bcl-2 to retard cell cycle entry of immortalized non-tumorigenic cell lines appears to be distinct from its role in apoptosis. Site-directed mutagenesis of tyrosine 28 in the BH4 domain of Bcl-2 markedly reduced its ability to restrain re-entry of quiescent cells in some context, but had no apparent effect on its anti-apoptotic activity [13]. These findings raise the possibility that Bcl-2 may also regulate cell cycle progression in adenocarcinomas, but the effect of Bcl-2 has not yet been examined in these cells. There is, however, some indirect evidence suggesting that the antiproliferative activity of Bcl-2 may be involved in tumorogenesis. In human breast and colorectal cancers, advanced adenocarcinomas have lost Bcl-2 expression, while elevated Bcl-2 levels has been associated with decreased proliferation and better prognosis [14,15]. In a mouse model for mammary tumor development, the ability of Bcl-2 to accelerate tumor progression correlated with a selective loss of its antiproliferative activity [16].

To investigate whether Bcl-2 regulates cell proliferation of human ovarian carcinoma cells, we took advantage of two isogenic cell lines, which expressed differential levels of Bcl-2 proteins. We show that the rate of cell proliferation is markedly reduced in Bcl-2-overexpressing SKOV3 cells compared to its low Bcl-2-expressing counterpart. This effect correlates with an accumulation of cells in S phase. Furthermore, SKOV3 cells in which Bcl-2 function is inhibited by scFvs display faster growth rates and cell cycle progression. Thus, the data presented here implicate Bcl-2 as a regulator of cell proliferation of human ovarian carcinoma cells. Furthermore, our data suggest, for the first time, that in addition to its known role at the G0/G1 checkpoint, Bcl-2 may also regulate the G2/M cell cycle checkpoint.

Materials and methods

Reagents and cell culture

The SKOV3.ip1 human epithelial ovarian cancer cell lines were kindly provided by J. Price (MD Anderson Cancer Center, Houston, TX). The SKOV3 cell line (human ovarian carcinoma) was obtained from the American Type Culture Collection (Manassas, VA). Both SKOV3 and SKOV3.ip1 cell lines are p53 null. All cell lines were maintained at 37°C in a humidified incubator containing 5% CO_2 in DMEM/F12 (BioMedia) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (BioMedia) and antibiotics. *Cis*-diamminedichloroplatinum (cisplatin), blasticidin, and doxycycline were all obtained from Sigma Canada Ltd. (Oakville, Ontario, Canada). Anti-human c-*myc* antibody was purchased from Santa Cruz Biotechnology Inc. Anti-Bcl-2 antibody was obtained from DAKO and anti-tubulin from Sigma. Anti-Bcl-X_L antibody was from Cell Signaling Technology (Berkeley, MA). HRP-conjugated anti-mouse, rabbit, or goat antibodies were purchased from Jackson Immuno Research Laboratories.

Plasmid constructs and transfection

Plasmid pLTR.Bcl-2 scFv was generated by PCR using a pSTCF.cyto Bcl-2 scFv template [19] and *Xho*I and *Not*I primers. After digestion of the PCR product at the *Xho*I and *Not*I sites, resulting fragments were cloned into *Xho*I/*Not*I-digested pLTR.linker described previously [17]. The resulting plasmid, pLTR.Bcl-2 scFv, is derived from the Moloney murine leukemia virus retroviral vector and contains the reverse tetracycline controlled transactivator (rtTA), an internal ribosome entry site (IRES), the blasticidin S deaminase (BSD) sequence, and the full-length anti-Bcl-2 scFv cDNA fused to a c-*myc* epitope-tag under the control of the doxycycline inducible minimal human cytomegalovirus immediate early promoter (tetO-CMV). In the absence of doxycycline, the transactivator does not recognize its specific DNA target sequence (tetO) and therefore transcriptional activation of scFvs does not occur. The addition of doxycycline results in binding of rtTA to tetO, which activates anti-Bcl-2 scFv transcription. The doxycycline is added at the concentration of 1 μg/ml as previously described with this system [18]. pRcCMV/Bcl-2 expression vector, kindly provided by J. Reed (The Burham Institute, CA), contains the human Bcl-2 cDNA under the control of the CMV promoter. pcDNA3/Bax expression vector was provided by L. Bouchard (Telogene, Sherbrooke, Canada). It contains the full-length human Bax cDNA fused to a HA epitope-tagged under the control of the CMV promoter.

Transfection of SKVO3 cells to generate stable clones was performed with pLTR.Bcl-2 scFv or empty control vector, pLTR.linker, using Fugene (Invitrogen), according to the manufacturer's recommendations. Stable transfectants were selected in the presence of blasticidin (1 μg/ml). Approximately 2 weeks later, independent clones were isolated, amplified, and Western blotted for expression of anti-Bcl-2 scFv using an anti-c-*myc* antibody. Alternatively, fluorescence images were obtained at 48 h after the addition of doxycycline (1 μg/ml) and incubation fluorescein-conjugated anti-c-*myc* antibody using a confocal immunofluorescent microscope (model IX-70, Olympus, Tokyo, Japan).