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Z-FL-COCHO, a cathepsin S inhibitor, enhances oxaliplatin-induced apoptosis through upregulation of Bim expression

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ABSTRACT

Inhibition of cathespsin S not only inhibits invasion and angiogenesis, but also induces apoptosis and autophagy in cancer cells. In present study, we revealed that pharmacological inhibitor [Z-FL-COCHO (ZFL)] of cathepsin S up-regulates pro-apoptotic protein Bim expression at the posttranslational levels. These effects were not associated with MAPKs and AMPK signal pathways. Interestingly, pretreatment with the chemical chaperones (TUDCA and PBA) and knockdown of protein phosphatase 2A (PP2A) markedly inhibited ZFL-induced Bim upregulation. ZFL enhances oxaliplatin-mediated apoptosis through ER stress-induced Bim upregulation in cancer cells. Collectively, our results suggest that inhibition of cathepsin S-induced Bim upregulation contribute to anti-cancer drug-induced apoptotic cell death in renal carcinoma Caki cells.

1. Introduction

The endoplasmic reticulum (ER) has a critical role in protein folding, protein modification, secretion, and calcium storage. Multiple cellular stress conditions relating to the accumulation of unfolded or misfolded proteins in the ER lumen leading to so-called ER stress [1–3]. ER stress has been reported to activate proapoptotic BH3-only protein Bim in various cancer cells [4–7]. Bim protein is upregulated by both transcriptional and posttranslational mechanisms in response to ER stress [4].

Cathepsin S is a cysteine protease of lysosome that is overexpressed in various malignant cancer cells [8–10]. Cathepsin S has been shown to possess an important role in angiogenesis and in modulating the major histocompatibility complex class II-dependent immune response [11,12]. Inhibition of cathepsin S by gene silencing or pharmacological inhibition [cathepsin S inhibitor; Z-FL-COCHO (ZFL)] induces autophagy, and an autophagy inhibitor reduces the ZFL-induced apoptosis in glioblastoma cells [13]. Furthermore, cathepsin S inhibition enhances TRAIL-induced apoptosis, downregulation of Bcl-2 and c-FLIP is associated with combined treatment-induced apoptosis [14]. Although it has been reported that cathepsin S is critically involved in tumor progression, the relationship between cathepsin S and ER stress is still unclear.

In present study, for the first time, we provide mechanistic evidence that cathepsin S inhibition enhances oxaliplatin-induced apoptosis through up-regulation of Bim expression in human renal carcinoma Caki cells.

2. Materials and methods

2.1. Cells and materials

American Type Culture Collection supplied human cancer cells [renal carcinoma cells (Caki, ACHN, and A498), lung carcinoma cells (A549), and breast cancer cells (MDA-MB-231)] (Manassas, VA, USA) and cells were cultured using DMEM or RPMI containing 10% FBS, 20 mM HEPES buffer, and 100 μg/mL gentamycin. The lines were authenticated by standard morphological examination using microscopy. Calbiochem (San Diego, CA, USA) supplied Z-FL-COCHO, and Sigma Chemical Co. (St. Louis, MO, USA) supplied anti-actin antibody, oxaliplatin, tauroursodeoxycholic acid, 4-Phenylbutyric acid, compound C, calyculin A, okadaic acid, and brefeldin A. Plasmid pEGFP-HSP70 was a gift from Lois Greene (Addgene plasmid # 15215) [15]. Bim antibody was purchased from BD Biosciences (San Jose, CA, USA). Santa Cruz Biotechnology (Dallas, TX, USA) supplied antibodies, such as anti-cathepsin S, *anti-*

Abbreviations: ER, endoplasmic reticulum; ZFL, Z-FL-COCHO; PP2A, protein phosphatase 2A; PARP, population and poly (ADP-ribose) polymerase; CHX, cycloheximide; AMPK, AMP-activated protein kinase.

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AMPKα1/2 and *anti*-ATF4, and Cell Signaling Technology (Beverly, MA, USA) supplied *anti*-PARP, *anti*-phospho-AMPKα anti-CHOP, and *anti*-REDD1 antibodies. Enzo life science (Farmington, NY, USA) supplied PD098059, SB203580, SP600125, and *anti*-GRP78 antibody. EDM Millipore (Darmstadt, Germany) supplied anti-PP2A antibody.

2.2. Small interfering RNA (siRNA)

Santa Cruz Biotechnology supplied the cathepsin S, AMPK, and PP2A siRNA (Dallas, TX, USA), and control [green fluorescent protein (GFP)] siRNA was purchased from Bioneer (Daejeon, Korea). The siRNA oligonucleotides transfected using LipofectamineTM RNAiMAX Reagent (Invitrogen, Carlsbad, CA, USA).

2.3. Western blot analysis

We made whole cell lysates using modified RIPA buffer as mentioned in previous our study [14,16,17]. We detected specific proteins using an enhanced chemiluminescence (ECL) Western blotting kit according to the manufacturer's instructions (WBKLS0500; Millipore Corp, Bedford, MA, USA).

2.4. Flow cytometry analysis

Cell counts were performed using a hemocytometer. After drug treatment, approximately 0.1×10^6 cells were suspended in 100 μL of PBS, and then 200 μL of 95% ethanol added into tube. The cells were maintained at 4 °C, after 1 h, cells were washed with PBS, and then resuspended in 250 μL of 1.12% sodium citrate buffer (pH 8.4) supplemented with 12.5 μg of RNase. For reaction, cells kept at 37 °C for 30 min, cellular DNA was stained using 250 μL of propidium iodide (50 μg/mL) for 30 min at room temperature. The stained cells were analyzed by fluorescent activated cell sorting (FACS) on a FACS Canto™ (BD Biosciences, San Diego, CA, USA).

2.5. Reverse transcription polymerase chain reaction (RT-PCR)

We isolated total RNA using the TriZol reagent (Life Technologies; Gaithersburg, MD) and made the cDNA using M-MLV reverse

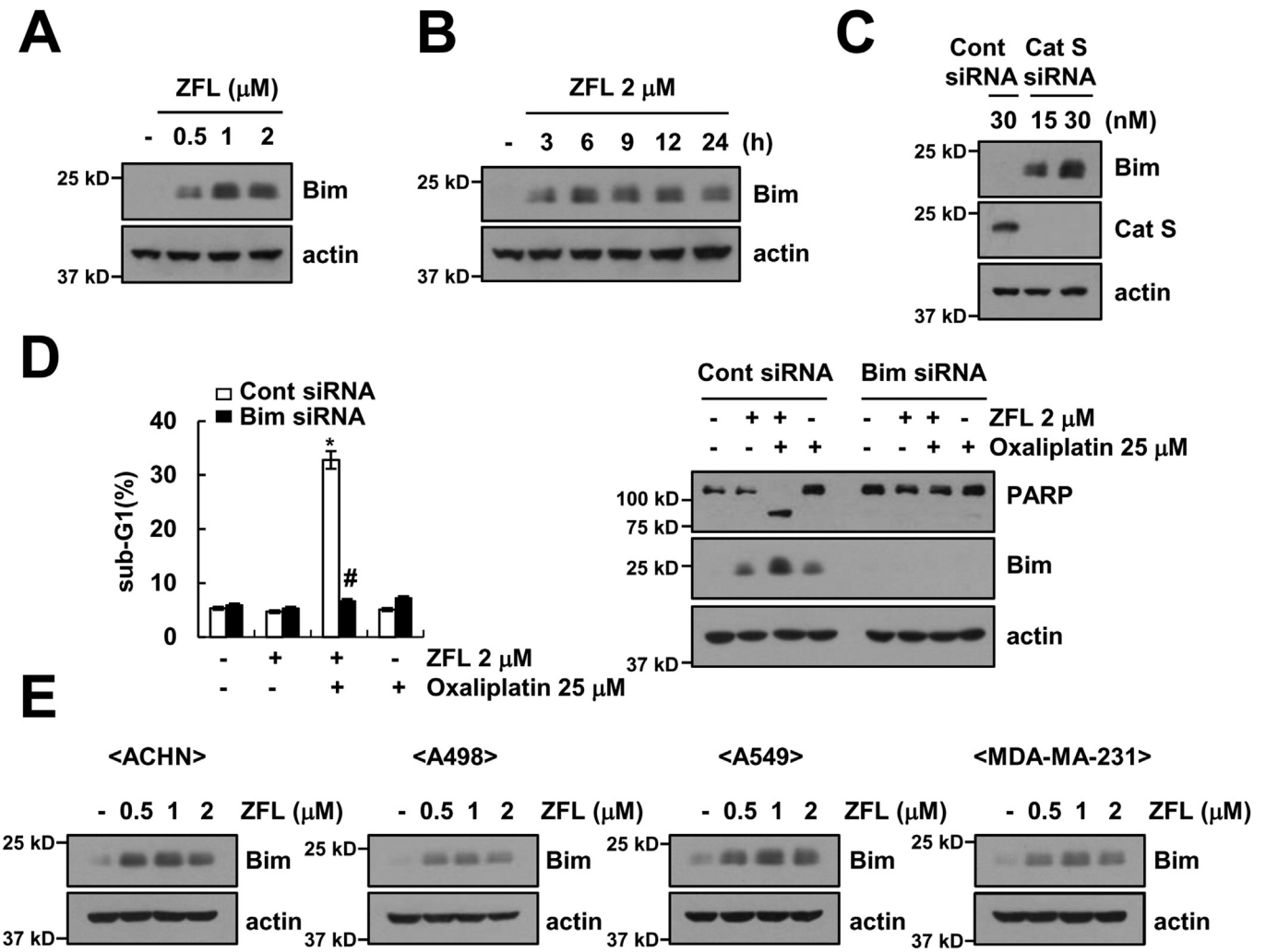

Fig. 1. ZFL induces Bim expression in cancer cells. (A) Caki cells were treated with various concentrations of ZFL for 24 h. (B) Cells were treated with 2 μM ZFL for the indicated time points. (C) Caki cells were transfected with siRNA against control or cathepsin S for 24 h. (D) Caki cells were transiently transfected with siRNA against control or Bim. After transfection, cells were treated with 2 μM ZFL plus 25 μM oxaliplatin for 24 h. Flow cytometry was used to detect the sub-G1 population. (E) Human cancer cells [renal (ACHN and A498), lung (A549), and breast (MDA-MB-231) carcinoma] were treated with 0.5, 1, or 2 μM ZFL for 24 h. Western blotting was used to detect the protein expression levels of Bim, cathepsin S, PARP, and actin. The values in D represent the mean ± SD of three independent samples. *$p < 0.01$ compared to control. #$p < 0.01$ compared to ZFL plus oxaliplatin-treated control siRNA s.

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