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ATF5 increases cisplatin-induced apoptosis through up-regulation of Cyclin D3 transcription in HeLa cells

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Abstract

ATF5 transcription factor plays an essential role in hematopoietic and glioma cell survival and neuronal cell differentiation. Here, we report for the first time the pro-apoptosis role of ATF5 and identify Cyclin D3 as an ATF5-targeted apoptosis-related gene. The ectopic expression of ATF5 in HeLa cells could markedly increase cisplatin-induced apoptosis and the cleavage of Caspase-3, and induce Cyclin D3 mRNA expression via cooperation with E2F1 transcription factor. Moreover, the interference of Cyclin D3 expression by transfection with Cyclin D3 RNAi could protect cells from ATF5-mediated apoptosis induced by cisplatin, indicating the contribution of Cyclin D3 in ATF5-mediated apoptosis. Taken together, these results suggest that ATF5 increases cisplatin-induced apoptosis through up-regulation of Cyclin D3 transcription, which elicits survival signals in HeLa cells.

Keywords: ATF5; Cyclin D3; Transcription; Apoptosis; Cisplatin; HeLa cells

Apoptosis, which is a common cellular response to stress caused by environmental challenges [1], may play a critical role in cancer chemotherapy [2]. To a great extent, many transcription factors, including E2F1 and c-Myc, play an important role in drug-induced apoptosis [3,4].

ATF5, a member of the activating transcription/cAMP response element binding protein (ATF/CREB) family, is a bZIP transcription factor [5], which could functionally and physically interact with a variety of proteins including Cyclin D3 [6], Tax [7], CHOP [8], and DISC1 [9]. To date, the anti-apoptosis role of ATF5 has been reported [10,11]. However, ATF5 could not block the apoptosis induced by DNA damage [10], indicating that the anti-apoptotic activity of ATF5 is selective. Consistently, retroviral delivery of a function-blocking mutant form of ATF5 into a rat glioma model evokes death of the infected tumor cells, but not of infected brain cells outside the tumors [11]. As mentioned above, it remains unclear whether ATF5 functions as a pro-apoptotic or anti-apoptotic factor in the process of apoptosis induced by DNA-damaging agent or which genes are regulated by ATF5 in this process.

In the present study, we show that the ectopic expression of ATF5 promotes cisplatin-induced apoptosis and up-regulates Cyclin D3 expression in HeLa cells. Moreover, interference of Cyclin D3 expression by transfection with Cyclin D3 RNAi could protect cells from ATF5-induced apoptosis. These results suggest that Cyclin D3 is an essential target gene of pro-apoptosis protein ATF5, which elicits survival signals in HeLa cells.

Experimental procedures

Materials. Restriction enzymes, bovine calf serum, DMEM, Trizol reagent, LipofectAMINE reagent, and the expression vectors pcDNA3.0, pcDNA3.1 were purchased from Invitrogen. PGL3-Basic and pRL-CMV were from Promega. Hoechst33258, dimethyl sulfoxide, cisplatin, and

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etoposide were purchased from Sigma Chemical. The anti-ATF5 Ab was purchased from Abcam. The anti-Cyclin D3 Ab, anti-Cyclin D2 Ab, and anti-Cyclin D1 Ab were purchased from Pharmingen. The anti-E2F1 Ab and anti-GAPDH Ab were purchased from Santa Cruz Biotechnology. The anti-cleaved Caspase 3 Ab was purchased from Cell Signal.

Cell culture and cell transfections. HeLa cells were cultured in Dulbecco's modified Eagle's medium. Cell transfection was performed with Lipofectamine (Invitrogen) according to the manufacturer's instructions.

Analysis of apoptosis by fluorescence staining, flow cytometry, and Western blot analysis. Fluorescence staining and flow cytometry have been described previously [12]. Western blots were performed as previously described [12], using an antibody to GAPDH to ensure equivalent loading.

Plasmids. A 2000-bp fragment (containing nucleotides from −2064 to −1) of Cyclin D3 promoter was prepared by PCR amplification of human genomic DNA using a sense primer containing a *Kpn*I restriction site and an antisense containing an *Xho*I restriction site. Primers were synthesized on the basic of the reported genomic sequence of Cyclin D3, forward 5′-GC GGGTACCATTCAGCAGGAAAATTTG-3′, and reverse 5′-AATCTC xGAGACTCGGGCAGCGAACA-3′. Following digestion with restriction enzymes, the Cyclin D3 promoter fragment was directionally cloned into *Kpn*I/*Xho*I digested pGL3Basic vector to generate a "full-length" Cyclin D3 promoter. Reporter genes containing sequentially truncated fragments (−1562/−1, −1002/−1, −573/−1, −162/−1, and −135/−1) of the Cyclin D3 promoter region were prepared in a similar manner. To prepare E2F1-binding site mutated promoter, the putative E2F1 transcription factor binding site CGCGC between nucleotide position −139 and −135 was changed to ATAAT and named M (E2F1). The mutation was created from Cyclin D3-Luc (−162 bp) by PCR using TakaRa MutantBEST mutagenesis kit. The pcDNA3.0-hATF5 plasmid has been described [6]. The pcDNA3.1-hATF1 plasmid was obtained by PCR amplification of pGFL-ATF1 (a gift from Dr. Gunther Schutz) and the PCR products were digested and inserted into pcDNA3.1-myc. The primers used were forward 5′-GTAGATT CATGGAAGATTCCCACAAGAGTA-3′ and reverse 5′-CAGGTAC CAACACTTTTATTGGAATAAAGATC-3′. The entire open reading frames of human ATF2, ATF3, and ATF4 genes were obtained by PCR amplification of the human fetal brain cDNA library (Clontech) in a similar manner. The construction for Cyclin D3 and Cyclin D3 RNAi plasmids has been described [13].

Dual luciferase assay and reverse transcription (RT)-PCR. Dual luciferase assay and reverse transcription PCR were performed as described [14]. Primers used for PCR were as follows: Cyclin D3-F 5′-AGTTGCGGGACTGGGAGGTG-3′ and Cyclin D3-R 5′-GTAGCAC AGAGGGCCAAAA-3′. The PCR products for Cyclin D3 were 184 bp.

Results

Ectopic expression of ATF5 increases apoptosis induced by Cisplatin in HeLa cells

To elucidate the role of ATF5 in the DNA damage agent-induced apoptosis, ATF5 expression vector or empty vector was transfected into HeLa cells (Fig. 1A). Next, we investigated the effect of ATF5 on apoptosis after cisplatin treatment for 12 h. As shown in Fig. 1B, ATF5 sensitized HeLa cells to cisplatin-induced apoptosis as indicated by fragmented and condensed nuclei, indicating the pro-apoptotic role of ATF5 in HeLa cells. This conclusion was further supported in Figs. 1C and D. The percentage of apoptotic cells in ATF5 overexpression cells was markedly increased compared to

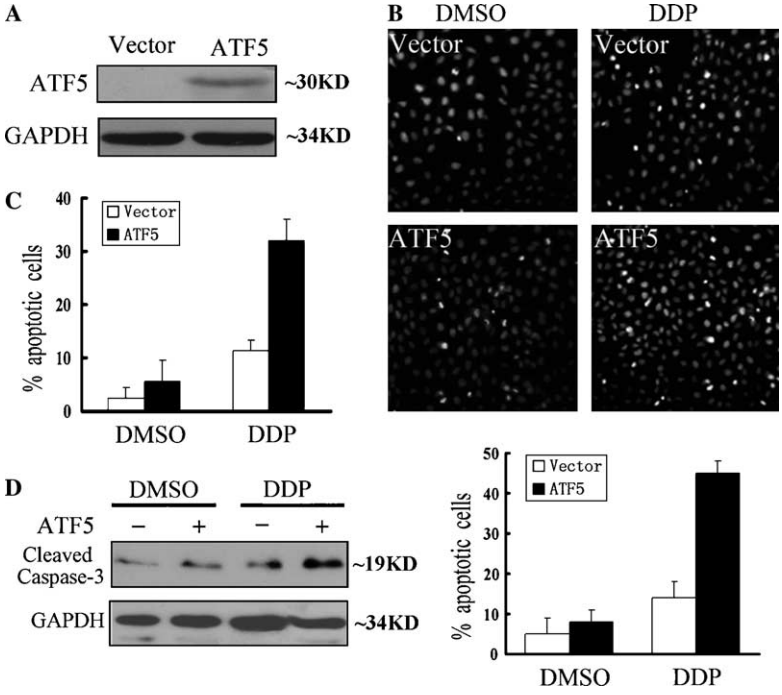

Fig. 1. The ectopic expression of ATF5 increases apoptosis induced by cisplatin (DDP) in HeLa cells. (A) HeLa cells were transiently transfected with control vector or ATF5 expression plasmid and cell extracts were analyzed by immunoblotting with an anti-ATF5 Ab. GAPDH served as a loading control. (B) Apoptosis was assessed morphologically. Hoechst 33258 staining of nuclei from cells transiently transfected with the empty vector or ATF5 in the absence or presence of DDP (5 μg/ml) for 12 h (upper). At least 300 cells were counted from three different microscope fields and each value was the mean ± SD of three independent experiments (lower). (C) Cells transfected with the empty vector or ATF5 expression vector were harvested after the treatment of DMSO or DDP (5 μg/ml), fixed in ethanol, and stained with propidium iodide. The apoptotic rates were counted by flow cytometry analysis ($n = 3$; $p < 0.05$). (D) Western blot analysis of Caspase-3 processing in total cell extracts of HeLa cell transfection with either empty vector or ATF5 expression vector treated with DMSO or DDP (5 μg/ml) for 12 h.