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Myocardin-related transcription factor A (MRTF-A) mediates doxorubicin-induced PERP transcription in colon cancer cells

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ABSTRACT

Doxorubicin (DOX) is a cytotoxic compound capable of instigating apoptosis in cancer cells. TP53 apoptosis effector (PERP) is a key mediator of apoptosis in multiple cell types. PERP transcription is activated by a range of pro-apoptotic stimuli. In the present study, we investigated the regulation of DOX-induced PERP transcription in colon cancer cells (SW480) by the transcriptional modulator myocardin-related transcription factor A (MRTF-A). We report that DOX treatment up-regulated MRTF-A expression paralleling PERP activation. DOX also promoted nuclear translocation of MRTF-A. On the contrary, MRTF-A depletion or inhibition attenuated DOX-induced apoptosis as evidenced by the MTT assay and caspase 3 cleavage. In accordance, MRTF-A depletion or inhibition dampened PERP transcription. Chromatin immunoprecipitation (ChIP) assay showed that DOX treatment promoted the binding of MRTF-A on the PERP promoter. Mechanistically, MRTF-A was recruited to the PERP promoter by activator protein 1 (AP-1). AP-1 interacted and cooperated with MRTF-A to activate PERP transcription. AP-1 silencing weakened PERP trans-activation by DOX presumably by compromising MRTF-A recruitment to the PERP promoter. In conclusion, our data suggest that MRTF-A might be a key regulator of DOX-induced PERP transcription in colon cancer cells.

1. Introduction

Cancers pose a serious health risk to humans with significant morbidity and mortality creating a heavy socioeconomic burden [1]. Despite rigorous research efforts by the academic and the industrial/pharmaceutical sectors, cancer-free survival rates for certain types of cancers have remained stagnantly low, indicating that there exist huge gaps in the current understanding of cancer pathobiology [2]. During carcinogenesis, cancer cells can be differentiated from their normal counterparts by acquiring several distinctive characteristics including anchorage-independent growth, uncontrolled proliferation, evasion of immune surveillance, augmented migration and invasion, and increased adaptability to “foreign soil” [3]. These newly acquired characteristics cumulatively bestow a significant advantage of survival on cancer cells such that these cells become immortalized and drive the normal cells out of existence. As such, one of the anti-cancer therapeutic tactics is to deprive the cancer cells of this advantage by forcing them to enter programmed cell death [4].

Programmed cell death, or apoptosis, is a tightly and intricately regulated process conserved during evolution [5]. During development, apoptosis functions to help lineage specification and differentiation; postnatally, apoptosis participates in both physiological and pathophysiological processes [6]. Cellular apoptosis is programmed by a network of factors, which can be roughly categorized as pro-apoptotic and anti-apoptotic. TP53 apoptosis effector (PERP) is a pro-apoptotic protein initially characterized by Tyler Jacks and colleagues as a transcriptional target for p53 in mouse embryonic fibroblasts (MEFs) in response to DNA damage [7]. Further characterization of this protein has revealed that PERP is a trans-membrane protein that possesses both apoptosis-dependent and -independent functions [8]. Unlike many other pro-apoptotic proteins localized to the mitochondria, PERP

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may promote apoptosis in a non-canonical manner likely through shipping intracellular death receptors to the cell membrane [9]. PERP levels are up-regulated by a plethora of pro-apoptotic stimuli in different cell types primarily at the transcriptional level although the underlying mechanism is not completely elucidated.

Myocardin-related transcription factor A (MRTF-A) is a transcriptional co-factor with ubiquitous expression patterns [10]. Mounting evidence suggests that MRTF-A may play diverse roles in carcinogenesis by modulating cancer cell migration/invasion [11,12], proliferation [13], senescence [14], and apoptosis [15]. In the present study we investigated the involvement of MRTF-A in the regulation of PERP transcription induced by doxorubicin (DOX), a DNA intercalating reagent commonly used in cancer chemotherapy, in cultured colon cancer cells. Our data suggest that MRTF-A can mediate DOX-induced PERP transcription by interacting with activator protein 1 (AP-1).

2. Methods

2.1. Cell culture and transient transfection

SW480 cells were maintained in RAPI1640 supplemented with 10% fetal bovine serum [16]. Expression constructs and the PERP promoter luciferase constructs have been previously described [17,18]. Small interfering RNAs were purchased from Dharmacon. Transient transfections were performed with Lipofectamine 2000 (Invitrogen).

2.2. MTT assay

MTT assay was performed using a commercially available kit (Jiancheng Biotech) according to manufacturer's recommendation. Briefly, MTT solution (5 mg/ml) was added to and incubated the cells for 4 h. Subsequently, the Formazan solution was added to and incubated with the cells for additional 4 h. Absorbance was measured at 570 nm.

2.3. Immunofluorescence microscopy

Immunofluorescence staining was performed essentially as described previously [19]. Cells were fixed with 4% formaldehyde, permeabilized with TBST, blocked with 5% BSA, and incubated with anti-MRTF-A (Proteintech) overnight. After several washes with PBS, cells were incubated with FITC-labeled secondary antibodies (Jackson) for 30 min. DAPI (Sigma) was added and incubated with cells for 5 min prior to observation. Immunofluorescence was visualized on a co-focal microscope (LSM 710, Zeiss).

2.4. Protein extraction and Western blot

Whole cell lysates were obtained by re-suspending cell pellets in RIPA buffer (50 mM Tris pH7.4, 150 mM NaCl, 1% Triton X-100) with freshly added protease inhibitor (Roche). Western blot analyses were performed with anti-MRTF-A (Santa Cruz), anti-PERP (Proteintech), anti-c-Jun (Santa Cruz), anti-c-Fos (Santa Cruz), anti-Lamin B (Proteintech), anti-β-actin (Sigma), and anti-α-tubulin (Proteintech) antibodies.

2.5. RNA isolation and real-time PCR

RNA was extracted with the RNeasy RNA isolation kit (Qiagen). Reverse transcriptase reactions were performed using a Super-Script First-strand Synthesis System (Invitrogen). Real-time PCR reactions were performed on an ABI Prism 7500 system. Primers and Taqman probes used for real-time reactions were purchased from Applied Biosystems.

2.6. Chromatin immunoprecipitation (ChIP)

Chromatin Immunoprecipitation (ChIP) assays were performed essentially as described before [20,21]. Aliquots of nuclear lysates containing 100 μg of protein were used for each immunoprecipitation reaction with anti-MRTF-A (Santa Cruz), anti-c-Jun (Santa Cruz), anti-c-Fos (Santa Cruz), anti-H3K4Me3 (Millipore), anti-H3K9Me2 (Millipore), anti-ASH2 (Bethyl Laboratories), and anti-JMJD1A (Proteintech). Precipitated genomic DNA was amplified by real-time PCR with the following primers: *PERP* promoter, 5′-ACCAGCGGTTCTTCCTTCAA-3′ and 5′-ATCTCACCCATCTGCCTGGT-3′ and *GAPDH* promoter, 5′-GGGTTCCTATAAATACGGACTGC-3′ and 5′-CTGGCACTGCACAAGAAGA-3'.

2.7. Statistical analysis

One-way ANOVA with post-hoc Scheffe analyses were performed using an SPSS package. Data are presented as mean ± SD. P values smaller than 0.05 were considered statistically significant (*).

3. Results

3.1. Doxorubicin activates MRTF-A in SW480 cells

We started off by assessing the effects of doxorubicin (DOX) treatment on MRTF-A in SW480 cells. Quantitative PCR assay showed that exposure of SW-480 cells to DOX treatment elicit significant augmentation of MRTF-A message levels as early as 12 h; MRTF-A expression levels continued to rise throughout the course of treatment paralleling DOX-induced cell death as measured by the MTT assay (Fig. 1A). Western blotting confirmed that MRTF-A proteins levels in SW480 cells were up-regulated by DOX treatment mirroring an increase in cleaved caspase 3 levels, another indicator of apoptosis (Fig. 1B).

MRTF-A activity is modulated in part by nuclear import/export [10]. Prior to DOX treatment, more MRTF-A was found in the cytoplasm (~75%) than in the nucleus (~25%) in SW480 cells as evidenced by immunofluorescence staining. DOX treatment induced an appreciable shift of MRTF-A from the cytoplasm to the nucleus 24 h after the cells were exposed to DOX. By 48 h after the addition of DOX, MRTF-A became predominantly nuclear (Fig. 1C). Likewise, cell fractionation and Western blotting showed that MRTF-A trans-localized from the cytoplasm to the nucleus upon DOX stimulation in SW480 cells (Fig. 1D).

3.2. MRTF-A deficiency or inhibition attenuates cell apoptosis

Having observed that MRTF-A activation coincided with DOX-induced apoptosis, we proposed that MRTF-A might play a role in this process. We performed the following experiments to test this hypothesis. DOX treatment induced marked cell death as measured by the MTT assay; small interfering RNA mediated knockdown of MRTF-A significantly attenuated cell death (Fig. 1E). Similarly, cleaved caspase 3 levels were up-regulated in SW480 following DOX treatment; the up-regulation of cleaved caspase3 was partially blocked by MRTF-A silencing (Fig. 1F).

Next, we treated the cells with CCG-1423, a small-molecule compound reported to inhibit MRTF-A activity [22]. As shown in Fig. 1G, CCG-1423 treatment resulted in significant preservation of cell viability after DOX addition as measured by the MTT assay. Western blotting also showed that CCG-1423 treatment ameliorated the induction of caspase 3 cleavage by DOX in SW480 cells

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