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Downregulation of stathmin expression is mediated directly by Egr1 and associated with p53 activity in lung cancer cell line A549

Lin Fang [a,1], Long Min [a,1], Yan Lin [a,1], Gao Ping [b], Wang Rui [a], Zhang Ying [a], Wang Xi [a], He Ting [a], Liu Li [a], Dong Ke [a], Ren Jihong [a], Zhang Huizhong [a,*]

[a] Department of Clinical Laboratory and Research Center, Tangdu Hospital, Fourth Military Medical University, Xinsi Road, Xi'an, Shaanxi Province 710038, China
[b] Department of Gynecology and Obstetrics, Tangdu Hospital, Fourth Military Medical University, Xi'an, China

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ABSTRACT

Stathmin is overexpressed in a variety of assessed human malignancies and is correlated with tumor progression and poor prognosis. Downregulation of its expression will contribute to optimize therapeutic outcomes in the treatment of various malignancies. However, the mechanisms of stathmin gene overexpression are not completely elucidated at present. Early growth response 1 (Egr1) is a transcription factor that triggers transcription of downstream genes mediating cell growth and angiogenesis upon various stimulations. Following the previous computational identification of a site that was thought to be an Egr1 consensus binding sequence at −85 to −94 region in stathmin gene promoter, we analyzed the role of Egr1 in the regulation of stathmin gene expression in lung cancer cell line A549. The results showed that Egr1 transcription factor bound to the sequence 5′-GCGGGGGCG-3′ within human stathmin gene promoter; and in reporter gene assays and overexpression experiments, both stathmin gene promoter activity and stathmin gene expression level were downregulated following endogenous or exogenous expression of Egr1. Using wild type Egr1 and knockout Egr1 cell lines, we demonstrated that p53 negatively regulates stathmin expression through Egr1 pathway. In summary, Egr1 is a novel regulator of stathmin expression and p53 mediates the transcriptional repression of stathmin by Egr1 in human lung cancer cells.

1. Introduction

Stathmin is a major cytosolic phosphoprotein that plays a crucial role in the control of cellular division and proliferation by regulating the dynamics of the microtubules that make up the mitotic spindle [1–3]. Stathmin is expressed at high levels in a wide variety of human malignancies including lung cancer, uterine cervix cancer, bladder cancer, breast cancer, osteosarcoma, showing that the overexpression of stathmin gene plays an important role in maintenance of malignant phenotype in human malignancies [4–6]. Our previous studies on stathmin have shown that inhibition of stathmin expression in malignant cells can abolish their transformed phenotype [7–9], however, why and how stathmin is overexpressed in a lot of human malignancies is not fully understood. One strategy is to investigate the transcriptional regulation of stathmin gene expression.

Our computer-assisted analysis of the unique proximal promoter of human stathmin gene, which contains multiple GC-rich regions, identified a potential binding site for Egr1 transcription factor at −85 to −94 region. Egr1 preferentially binds to the GC-rich sequence 5′-GCGGGGGCG-3′ [10,11].

Egr1 (also called Zif268, Krox24, NGFI-A, Tis8) is a zinc-finger nuclear phosphoprotein and belongs to the family of cellular immediate early genes of transcription factor and is rapidly induced by various mitogens, growth factors, and ionizing radiation [12–14]. Phorbol 12-myristate-13-acetate (PMA) is a potent mitogen and can strongly upregulate Egr1 biosynthesis [15]. Egr1 is mainly regulated by posttranscriptional mechanisms, such as phosphorylation [16]. It has been shown that Egr1 stimulates the synthesis of growth and differentiation factor via direct promoter activation, and in this way, Egr1 acts as an important regulator of cell growth and differentiation [17–19]. In contrast to this growth stimulating function, Egr1 binds to the transcription factor c-Jun and increases the activity of c-Jun to promote cell apoptosis [20]. In our previous studies, we found that stathmin was overexpressed and Egr1 expression level was low in A549 cells (Fig. 1F and G).

The facts that stathmin gene promoter contains a potential binding site for Egr1 transcription factor by computational analysis and Egr1 can also be upregulated by tumor suppressor gene p53, which leads to p53-dependent apoptosis [21], and p53 is also involved in the negative regulation of stathmin gene expression [22], while there was no p53

Abbreviations: siRNA, small interfering RNA; CMV, cytomegalovirus; Neo, Neomycin; EMSA, Electrophoretic mobility shift assay; PMA, phorbol 12-myristate-13-acetate; ChIP, Chromatin immunoprecipitation.

* Corresponding author. Tel.: +86 29 84777470; fax: +86 29 84777470.
E-mail address: Zhz328@yahoo.com.cn (Z. Huizhong).

[1] Lin Fang, Long Min and Yan Lin should be regarded as joint first authors for equal contributions.

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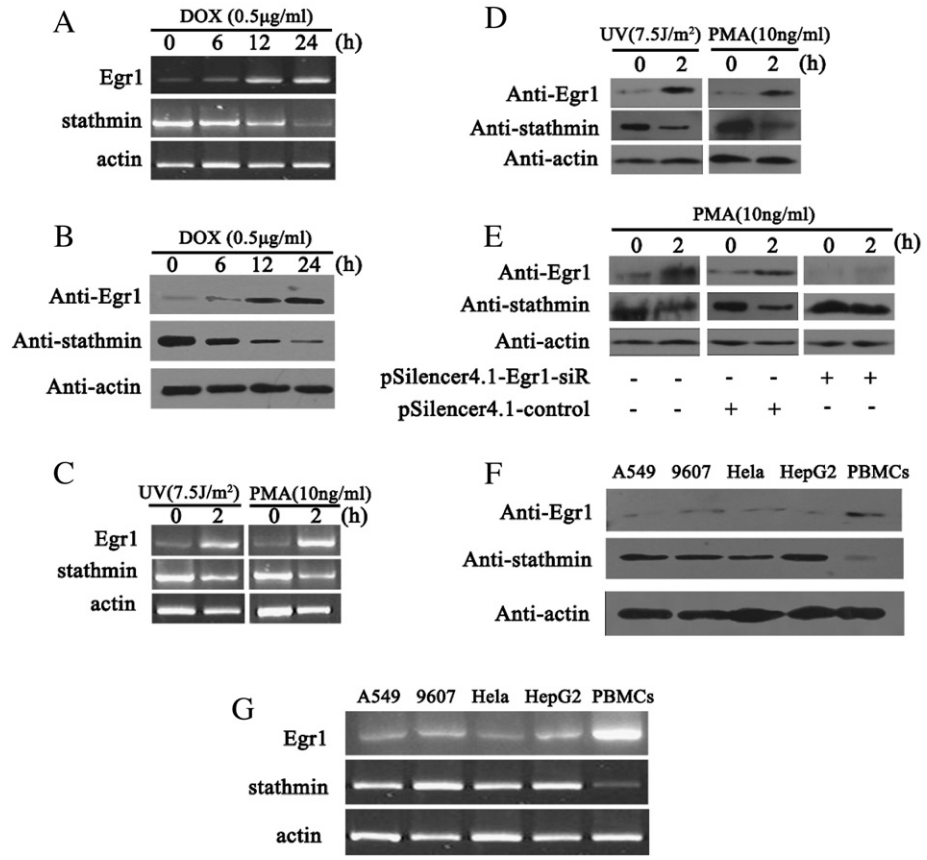

Fig. 1. Downregulation of stathmin by DNA-damaging reagents or stress is Egr1 dependent. (A, B, C and D) Egr1 and stathmin were assessed by RT-PCR and western blotting assays in A549 cells that were treated with DOX, UV or PMA. (E) A549 cells were transiently transfected with pSilencer4.1-Egr1-siR vector or pSilencer4.1-control vector. After 24 h, cells were treated with PMA. Egr1 and stathmin were assessed by western blotting. (F and G) Egr1 and stathmin were assessed by RT-PCR and western blotting in some human cell lines. Actin was used as a loading control. The experimental details are described in the Materials and methods section.

binding site in stathmin gene promoter using computational analysis, led us to question whether there is a relationship between stathmin gene expression and p53 regulation through Egr1, which would help identify the biological function of stathmin and better understand the p53-regulated cellular responses. Nevertheless, the complete regulation of stathmin gene expression is not fully understood, we investigated the regulation of stathmin gene promoter by Egr1 in this study, which may have significant implications for the functional activity of stathmin gene and reveal additional approaches for new cancer therapeutic strategies.

2. Materials and methods

2.1. Cells and culture conditions

Human lung cancer cell line A549 (wild type p53 and Egr1) and human embryonic kidney cell line 293 (HEK293) were cultured in Dulbecco′s Modified Eagle′s medium (Invitrogen, USA) supplemented with 10% fetal bovine serum and penicillin (100 U/ml)/streptomycin (0.1 mg/ml). All cells were maintained under the atmosphere of 5% CO_2 with humidity at 37 °C. Peripheral blood mononuclear cells (PBMCs) from healthy donors were isolated by gradient centrifugation (Sigma, USA), and were cultured in RPMI-1640 medium (Invitrogen, USA). Donors willing to participate were required to complete a written donor affidavit form, consenting to donate residue blood for research purposes. For treatment, cells were cultured in the presence of 10 ng/ml phorbol 12-myristate-13-acetate (PMA; Sigma, USA) or 0.5 μg/ml Doxorubicin (DOX; Sigma, USA).

2.2. UV treatment

Ultraviolet lamps (FS72T12-UVB-HO; National Biological Corp, USA) which emit UVB (290–320 nm; 75–80% of the total energy) and UVA (320–375 nm; 20–25% of the total energy) were used in all experiments. The medium was removed from the dishes and the cells were rinsed twice with phosphate-buffered saline (PBS) at 37 °C, drained, and irradiated with the indicated doses of UV. Fresh medium at 37 °C was added after the irradiation.

2.3. RT-PCR (reverse transcription polymerase chain reaction) analysis

Total cellular RNA was extracted using TRIzol reagent (Invitrogen, USA) and 5 μg of RNA was used to synthesize cDNA using Superscript First-Strand Synthesis Kit (Promega, USA) according to the manufacturer′s protocols. The following primers were used at the indicated annealing temperature: stathmin gene (AB451319) sense: 5′-ATGGCTTCTTCTGATATCCAG-3′ (1–21), and antisense: 5′-TTAGTCAGCTTCAG TCTCGTC-3′ (429–450) at 60 °C for 30 cycles, a predicted band was 450 bp; Egr1 (NM_001964) sense: 5′-AGCCCTACGAGCACCTGAC-3′ (554–572) and antisense: 5′-TGGGTTGG TCATGCTCACTA-3′ (752–771) at 62 °C for 30 cycles, a predicted band was 218 bp; β-actin (X00351) gene was used as an internal standard and was amplified with primers, sense: 5′-CTACAATGAGCTGCGTG-3′ (311–327), and antisense: 5′-GGTCTCAAACATGATC-3′ (404–419) at 58 °C for 30 cycles, a predicted band was 109 bp. Then RT-PCR products were electrophoresed through a 1.5% agarose gel with ethidium bromide. Signals were quantified by densitometric analysis