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Original article

Periostin contributes to arsenic trioxide resistance in hepatocellular carcinoma cells under hypoxia

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ABSTRACT

Hypoxia has been suggested to induce chemoresistance in tumor cells. In this study, we aimed to test the hypothesis that hypoxia-inducible factor-1alpha (HIF-1α)/periostin axis might promote arsenic trioxide resistance in hepatocellular carcinoma (HCC) cells under hypoxia. HCC cells were exposed to hypoxia and measured for periostin expression. Loss-of-function studies were done to assess the role of periostin in arsenic trioxide resistance. *In vivo* xenograft mouse studies were performed to determine the effect of periostin silencing on HCC susceptibility to arsenic trioxide. It was found that periostin expression was significantly increased in SMMC7721 and Hep3B HCC cells after hypoxic treatment. Depletion of HIF-1α blocked the upregulation of periostin induced by hypoxia. HCC cells under hypoxia displayed more resistant to arsenic trioxide than those under normoxia. Interestingly, downregulation of periostin re-sensitized hypoxic SMMC7721 and Hep3B cells to arsenic trioxide, which was accompanied by increased apoptosis. Luciferase reporter assay revealed that periostin overexpression enhanced HIF-1α-dependent transcriptional activity and induced the expression of vascular endothelial growth factor, Mcl-1, and Bcl-xL in SMMC7721 cells. Administration of arsenic trioxide resulted in a significant inhibition of SMMC7721 tumor growth. Notably, downregulation of periostin significantly enhanced the anticancer effect of arsenic trioxide against SMMC7721 tumors and reduced the percentage of Ki-67-positive proliferating cells. Taken together, periostin contributes to arsenic trioxide resistance in HCC under hypoxic microenvironment, which is likely associated with promotion of HIF-1α-dependent activation of survival genes. Targeting periostin may represent a promising strategy to improve arsenic trioxide-based anticancer therapy against HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is a highly prevalent malignancy worldwide. Most HCC patients are diagnosed at advanced stages and may thus be precluded from curative surgery [1]. Transarterial chemoembolization (TACE) and systematic chemotherapy are important treatment options for unresectable advanced HCCs [2]. Sorafenib, a multikinase inhibitor, has been approved to treat patients with advanced HCC, but only produces limited survival benefits [3]. Arsenic trioxide, which is the primary chemotherapeutic agent for acute promyelocytic leukemia (APL), also shows cytotoxic effects against a variety of solid tumors [4,5]. However, development of drug resistance limits chemotherapeutic efficacy against HCC [6].

There is increasing evidence for the link between hypoxic stimuli and chemotherapeutic resistance in tumor cells [7,8]. Hypoxia-inducible factor-1alpha (HIF-1α) is a key transcription factor participating in the induction of hypoxia response element (HRE)-containing genes [9]. Several anti-apoptotic genes survivin, Mcl-1, and Bcl-xL have been identified as the target genes of HIF-1α, whose activation facilitates cell adaptation to hypoxic stress [10]. HIF-1α is also responsible for triggering the expression of pro-angiogenic genes, in particular vascular endothelial growth factor (VEGF) [10]. Periostin is an extracellular matrix protein highly expressed in HCC tissues [11] and contributes to tumor cell survival under hypoxia [12,13]. It was reported that periostin can induce the expression of HIF-1α and VEGF in glioma cells [14]. HIF-1α is involved in the upregulation of periostin in hypoxic keloid fibroblasts [15]. These studies suggest a cross-talk between HIF-1α and periostin expression.

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It has been documented that overexpression of HIF-1α leads to the acquisition of arsenic trioxide resistance in HCC cells under normoxic conditions [16]. However, the mechanism by which hypoxic HCC cells develop resistance to arsenic trioxide is still elusive. In this study, we tested the hypothesis that HIF-1α/periostin axis may govern arsenic trioxide resistance in HCC cells under hypoxia.

2. Materials and methods

2.1. Cell culture and treatment

Human HCC cell lines (SMMC7721 and Hep3B) were obtained from the Cell Bank of China Science Academy (Shanghai, China) and maintained in Dulbecco's modified Eagle's medium (DMEM; PAA Laboratories, Pasching, Austria) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin (PAA) at 37 °C in a 5% CO_2 atmosphere. For hypoxia exposure, cells were given a constant gas mixture of 1% O_2, 94% N_2, and 5% CO_2 for 48 h. Cells cultured under normoxic conditions (21% O_2, 74% N_2, and 5% CO_2) were used as control. For arsenic trioxide treatment, cells were treated with different concentrations of arsenic trioxide (1–16 μM) under hypoxia or normoxia for 48 h.

2.2. Real-time PCR analysis

Total RNA from cells was isolated using RNAiso Plus (TaKaRa, Dalian, China) as per the manufacturer's instructions. RNA samples were reverse transcribed into cDNA using the QuantiTect Reverse Transcription Kit (Qiagen, Hilden, Germany) with random hexamers. Real-time PCR analysis of periostin, VEGF, Mcl-1, Bcl-xL, and β-actin mRNA levels was performed on the 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with the FastStart Universal SYBR Green Master (Roche, Mannheim, Germany). The PCR primers are shown in Supplementary Table S1.

2.3. Western blot analysis

Protein extracts from cells were prepared in radioimmunoprecipitation assay (RIPA) buffer (Thermo Fisher Scientific Inc., Waltham, MA, USA) containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and Protease Inhibitor Cocktail (Roche). Protein samples were resolved by sodium dodecyl-sulfate polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The following antibodies were used in this study: anti-periostin (ab172615, Abcam, Cambridge, MA, USA; 1:300), anti-HIF-1α (sc-13515, Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:500), and β-actin (sc-1615, Santa Cruz Biotechnology; 1:1000 dilution). Protein bands were visualized by enhanced chemiluminescence (Pierce, Rockford, IL, USA). Densitometric quantitation of signals was performed using Quantity One software (Bio-Rad, Hercules, CA, USA).

2.4. Plasmid construction and cell transfection

Short hairpin RNAs (shRNAs) targeting *HIF-1α* or *periostin* and negative control shRNAs were purchased from Santa Cruz Biotechnology. Full-length human *periostin* and *HIF-1α* cDNA

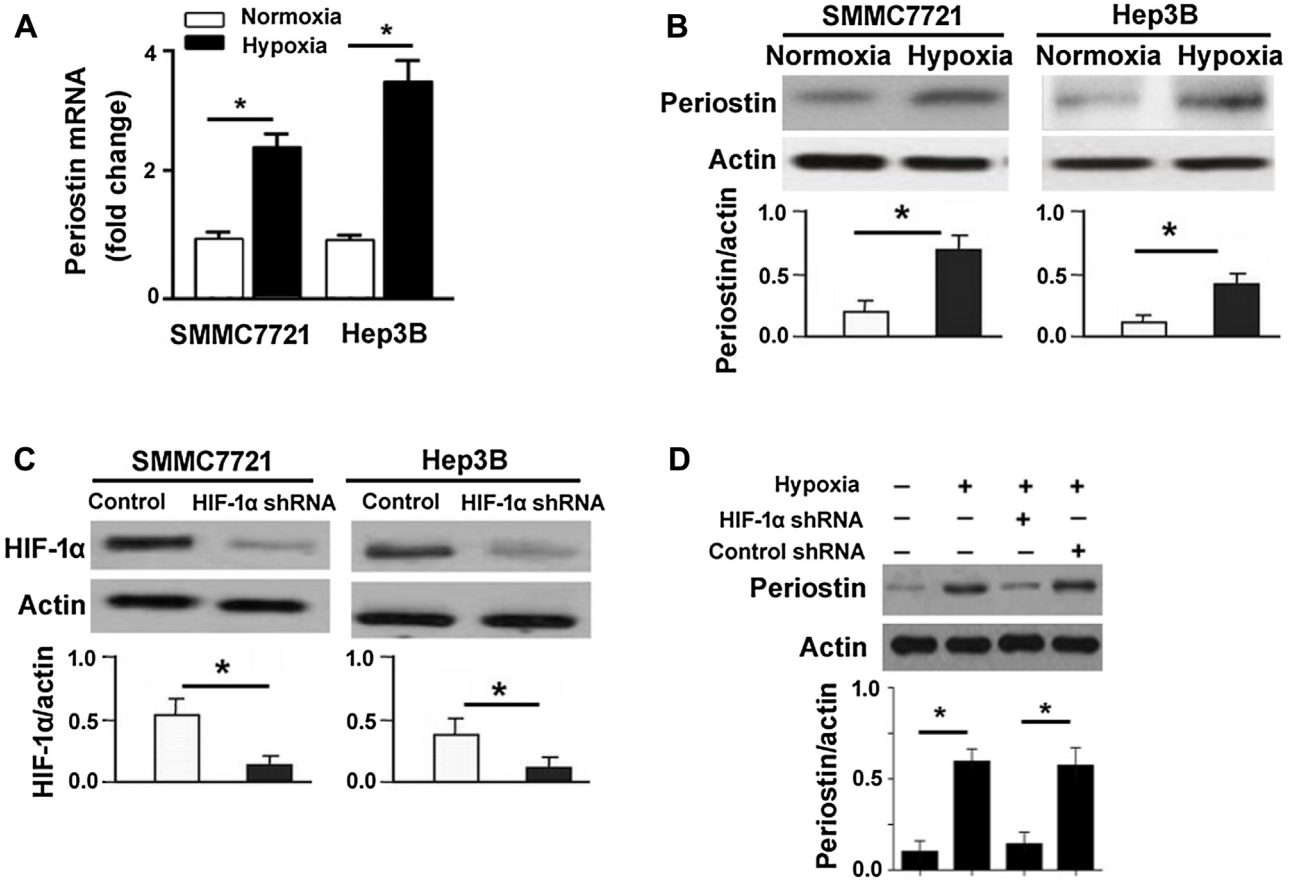

Fig. 1. Hypoxic stress induces the expression of periostin via a HIF-1α-mediated pathway. (A and B) SMMC7721 and Hep3B cells were exposed to hypoxia or normoxia and measured for periostin mRNA and protein levels by real-time PCR (A) and Western blot (B) analysis, respectively. (C) Western blot analysis of HIF-1α protein levels in SMMC7721 and Hep3B cells transfected with control or HIF-1α shRNA under hypoxic conditions. *Top*, representative Western blots. *Bottom*, quantitative data from three independent experiments. (D) Western blot analysis of periostin protein levels in HCC cells with indicated treatments. $^{*}P < 0.05$.