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Astragaloside IV inhibits cell migration and viability of hepatocellular carcinoma cells via suppressing long noncoding RNA ATB

Yaling Li*, Yun Ye, Hongyan Chen

Department of Pharmacy, The Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan, China

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ABSTRACT

Astragaloside IV (AS-IV), the major active component of *Astragalus membranaceus*, has shown attractive anticancer effects in certain cancers. However, the roles and action mechanisms of AS-IV in hepatocellular carcinoma (HCC) are largely unclear. Long noncoding RNAs (lncRNAs) are recently revealed to have crucial roles in HCC initiation and progression, but whether lncRNAs participate in the anticancer roles of AS-IV are unknown. In this study, we demonstrated that AS-IV significantly downregulated lncRNA-ATB expression in a dose- and time-dependent manner in HCC cells. Through downregulating lncRNA-ATB, AS-IV repressed epithelial-mesenchymal transition (EMT) and migration of HCC cells. Furthermore, through downregulating lncRNA-ATB, AS-IV inactivated IL-11/STAT3 signaling, induced HCC cell apoptosis, and decreased HCC cell viability. Overexpression of lncRNA-ATB reversed the effects of AS-IV on HCC cell migration, EMT, cell apoptosis, cell viability, and IL-11/STAT3 signaling. Taken together, our results showed that AS-IV inhibited migration and cell viability of HCC cells via downregulating lncRNA-ATB. Thus, our data provided a novel molecular basis for the applications of AS-IV in the therapy of HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most prevalent malignancy and the second leading cause of cancer-related death worldwide [1]. Surgical resection is still the primary therapeutic strategy for HCC [2]. However, most HCC patients may suffer postoperative recurrence and intrahepatic metastases due to the aggressive characteristics of HCC [3]. Unfortunately, most HCCs are not sensitive to current radiotherapy, chemotherapy, immunotherapy, and molecular targeted therapy [4,5]. Therefore, further revealing molecular mechanisms underlying HCC initiation and progression, and developing novel therapeutic agents for HCC are urgent [6,7].

Natural products have been used for many years to treat various diseases and have shown attractive therapeutic effects on a variety of cancers [8–12]. Astragaloside IV (AS-IV), the major active component of a kind of Chinese traditional herb *Astragalus membranaceus*, has a number of pharmacological activities, such as immunoregulatory, antioxidant, neuroprotective, anti-cancer, antiviral effects, and so on [13–16]. However, the pharmacological activities of AS-IV on HCC and the underlying action mechanisms are largely unknown.

Long noncoding RNA (lncRNA) is a class of transcripts with no protein coding potential and more than 200 nucleotides in length [17–20]. Increasing evidences have documented that the number of lncRNAs is great larger than that of mRNAs [21]. Furthermore, many lncRNAs have been revealed to be deregulated in many cancers and also many lncRNAs have critical roles in cancers initiation and development [22–27]. Long noncoding RNA activated by TGF-β (lncRNA-ATB) is first identified in HCC [28]. LncRNA-ATB is activated by TGF-β and upregulated in HCC. Functionally, lncRNA-ATB promotes epithelial-mesenchymal transition (EMT) and metastasis of HCC cells via competitively binding the miR-200 family, and also promotes survival of HCC cells via activating IL-11/STAT3 signaling. EMT is well known to endow cells with more migrated and invasive potential [29,30]. IL-11 is well known to activate STAT3 signaling and confer survival advantage to cancer cells [31]. Thus, lncRNA-ATB is regarded as an oncogenic lncRNA.

Transforming growth factor-β (TGF-β) is a multifunctional cytokine and has important roles in tumorigenesis and progression [32,33]. Intriguingly, Astragalus extracts have been reported to modulate TGF-β signaling in HCC [34,35]. Therefore, in this study we further investigated whether AS-IV could modulate the expression of lncRNA-ATB via the TGF-β signaling, and whether the oncogenic lncRNA-ATB mediated the roles of AS-IV in HCC.

* Corresponding author at: Department of Pharmacy, The Affiliated Hospital of Southwest Medical University, NO. 25 Taiping Street, Luzhou, Sichuan 646000, China.
E-mail address: liyalingswmu@yeah.net (Y. Li).

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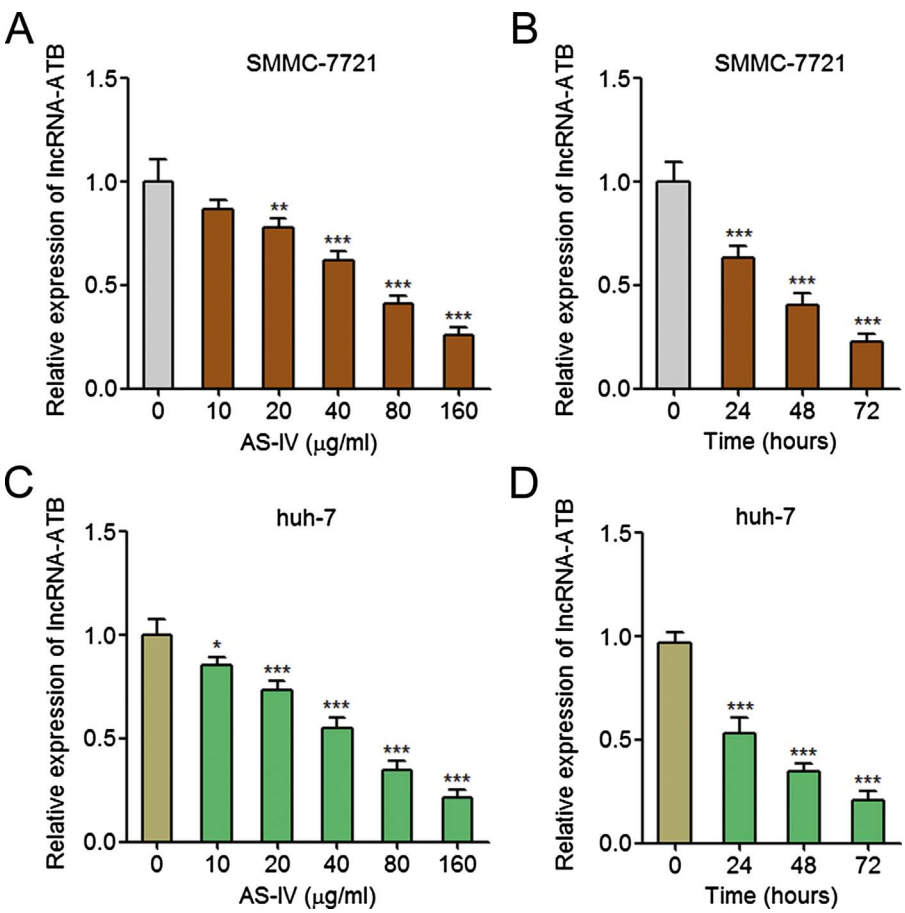

Fig. 1. AS-IV inhibits the expression of lncRNA-ATB in HCC cells. (A) lncRNA-ATB expression levels in SMMC-7721 cells treated with a series dose of AS-IV (0, 10, 20, 40, 80, 160 μg/ml) for 48 h were measured by qRT-PCR. (B) lncRNA-ATB expression levels in SMMC-7721 cells treated with 80 μg/ml AS-IV for 0, 24, 48, or 72 h were measured by qRT-PCR. (C) lncRNA-ATB expression levels in huh-7 cells treated with a series dose of AS-IV (0, 10, 20, 40, 80, 160 μg/ml) for 48 h were measured by qRT-PCR. (D) lncRNA-ATB expression levels in huh-7 cells treated with 80 μg/ml AS-IV for 0, 24, 48, or 72 h were measured by qRT-PCR. Data are shown as mean ± SD from 3 independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ by one-way ANOVA.

2. Materials and methods

2.1. Cell culture and treatment

The human HCC cell lines SMMC-7721 and huh-7 were obtained from Chinese Academy of Sciences Cell Bank (Shanghai, China). These cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Invitrogen) at 37 °C in a 5% CO2 incubator. AS-IV was obtained from J&K Scientific Ltd. (Beijing, China) and dissolved in DMSO at the concentration of 100 mg/mL and then stored at −20 °C for further dilution. Where indicated, cells were treated with AS-IV at indicated concentration for indicated time.

2.2. RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

When HCC cells grow to 80% confluence in 6-well culture-plates, indicated concentration of AS-IV was added for indicated time. Then, total RNA was extracted from these cells using TRIzol Reagent (Invitrogen) following the manufacturer's protocol. After being treated with DNase I (Takara, Dalian, Liaoning, China) to remove genomic DNA, 2 μg RNA was used to carry out reverse transcription with the M-MLV Reverse Transcriptase (Invitrogen) and either gene-specific primers or random primers. qRT-PCR was performed with SYBR® Premix Ex Taq™ II (Takara) on the StepOnePlus Real-time PCR system (Applied Biosystems, Foster City, CA, USA). GAPDH was used as endogenous control for the quantification of the expression of indicated lncRNAs and mRNAs. The expression of indicated lncRNAs and mRNAs was calculated using the $2^{-\Delta\Delta Ct}$ method. Primers sequences were as follows: lncRNA-ATB, 5′-ACACAGAATAAAATAACAC-3′ (reverse transcription), 5′-TCTGGCTGAGGCTGGTTGAC-3′ (sense), and 5′-ATCTCTGGGTGCTG GTGAAGG-3′ (antisense); E-cadherin, 5′-GCCCCATCAGGCCTCCG TTT-3′ (sense) and 5′-ACCTTGCCTTCTTTGTCTTTGTTGGA-3′ (antisense); N-cadherin, 5′-TGGACCATCACTCGGCTTA-3′ (sense) and 5′-ACACTGGCAAACCTTCACG-3′ (antisense); IL-11, 5′-GCTGCAAGGT CAAGATGGTT-3′ (sense) and 5′-GCTGGGTGGCGTTCTATC-3′ (antisense); and GAPDH, 5′-GGTCTCCTCTGACTTCAACA-3′ (sense) and 5′-GTGAGGGTCTCTCTCTTCCT-3′ (antisense).

2.3. Plasmids and stable cell lines construction

lncRNA-ATB expression vector was constructed as previously described [28]. Briefly, the cDNA encoding lncRNA-ATB was PCR amplified and subcloned into the *Hin*d III and *Eco*R I sites of pcDNA3.1 vector (Invitrogen) with the primers 5′-CTCAAGCTTGGCCCTGGGGCT CTGCAA-3′ (sense) and 5′-GGAATTCTGGTAAATGAGTCCAAAGTC-3′ (antisense). To obtain lncRNA-ATB stably overexpressed HCC cells, lncRNA-ATB expression vector was transfected into SMMC-7721 and huh-7 cells, and then SMMC-7721 and huh-7 cells were selected with 800 μg/ml or 1000 μg/ml neomycin for four weeks, respectively.

The oligonucleotides for shRNAs specifically targeting lncRNA-ATB were synthesized and inserted into the shRNA expression vector pGPH1/Neo (GenePharma, Shanghai, China). The two shRNAs target sequences for lncRNA-ATB were: 5′-CCTTATGGCCTAGATTACCTTT CCA-3′ and 5′-CCTGTCTGTATTTGCGAATACCTTT-3′. A scrambled non-silencing shRNA was used as negative control for lncRNA-ATB specific shRNAs. To obtain lncRNA-ATB stably depleted HCC cells, lncRNA-ATB specific shRNAs were transfected into SMMC-7721 and huh-7 cells, and then SMMC-7721 and huh-7 cells were selected with 800 μg/ml or 1000 μg/ml neomycin for four weeks, respectively.