Biomedicine & Pharmacotherapy 102 (2018) 302–308

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Sanguinarine inhibits epithelial ovarian cancer development via regulating long non-coding RNA CASC2-EIF4A3 axis and/or inhibiting NF-κB signaling or PI3K/AKT/mTOR pathway

Suxian Zhang, Tianyan Leng, Qin Zhang, Qinghua Zhao, Xiaofeng Nie, Lihua Yang*

Department of Gynaecology, The Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650101, China

ARTICLE INFO

Keywords:
Ovarian cancer
Sanguinarine
Long non-coding RNA CASC2
EIF4A3
Nuclear factor-κB signaling
PI3K/AKT/mTOR pathway

ABSTRACT

Objective: This study aimed to investigate the antitumor effects and possible regulatory mechanisms of sanguinarine in epithelial ovarian cancer.

Material and methods: The effects of sanguinarine on the malignant behaviors of epithelial ovarian cancer SKOV3 cells and the expression of long non-coding RNA CASC2 were investigated. The expression of CASC2 and EIF4A3 in epithelial ovarian cancer tissues and cells were detected, and the potential mechanisms of sanguinarine were explored by investigating the interactions between CASC2 and EIF4A3. Furthermore, the regulatory relationship between sanguinarine and nuclear factor-κB (NF-κB) signaling or PI3K/AKT/mTOR pathway was explored.

Results: Sanguinarine exhibited antitumor effects in SKOV3 cells by significantly inhibiting cell viability, migration and invasion and promoting cell apoptosis. Moreover, sanguinarine induced CASC2 expression and silencing of CASC2 reversed the effects of sanguinarine in epithelial ovarian cancer cells. CASC2 was significantly lowly expressed in ovarian cancer tissues and cells, while EIF4A3 was highly expressed. EIF4A3 was identified as a CASC2 binding protein. Knockdown of EIF4A3 reversed the effects of sanguinarine plus CASC2 silencing. Besides, sanguinarine markedly inhibited the activation of NF-κB signaling or PI3K/AKT/mTOR pathway, which was reversed by CASC2 silencing. And the effects of sanguinarine plus CASC2 silencing on the activation of these pathways were further reversed after knockdown of EIF4A3 at the same time.

Conclusions: Our findings reveal that sanguinarine exhibits antitumor effects in epithelial ovarian cancer cells possible via regulating CASC2-EIF4A3 axis and/or inhibiting NF-κB signaling or PI3K/AKT/mTOR pathway. Sanguinarine may serve as a potential therapeutic reagent for epithelial ovarian cancer.

1. Introduction

Ovarian cancer is the leading cause of gynaecological cancer-associated death [1], among which epithelial ovarian cancer comprises approximately 80% malignant ovarian neoplasms [2]. The prognosis of ovarian cancer is poor, with just 40% of patients surviving 5 years [3]. Despite great advances in surgical and chemotherapeutic options, it remains a challenge for the treatment of recurrent ovarian cancer [4]. Moreover, acquired resistance to chemotherapy is considered as a major limitation in the management of ovarian cancer [5]. Therefore, it is still imperative to design an effective therapeutic therapy for ovarian cancer.

Sanguinarine is a member of quaternary benzo[*c*]phenanthridine alkaloids (QBAs) that have many important properties, including antimicrobial, antifungal, antitumour and anti-inflammatory effects [6]. In non-small cell lung cancer, sanguinarine is found to play antitumor effect via upregulation of Fas-associated factor 1 [7]. It is also reported that sanguinarine can induce oxidative stress and inhibit sonic hedgehog-Gli-Nanog pathway to suppress the pancreatic cancer stem cell characteristics [8]; can regulate the DUSP4/ERK pathway to repress the growth and invasion of gastric cancer cells [9]; and can inhibit basal-like breast cancer growth via suppressing dihydrofolate reductase [10]. Nevertheless, the carcinogenic potential of sanguinarine has been reported in mouthwash-induced leukoplakia and gallbladder carcinoma [11]. Given these inconsistent results, whether sanguinarine functions antitumor activity or carcinogenic potential in ovarian cancer is largely unknown, let alone its regulatory mechanism.

Long non-coding RNAs (lncRNAs) are a class of RNAs with a length of > 200 nt that engage in numerous biological processes [12,13]. Accumulating studies have demonstrated that several lncRNAs are key

* Corresponding author at: Department of Gynaecology, The Second Affiliated Hospital of Kunming Medical University, NO. 374 Dianmian Avenue, Wuhua District, Kunming, Yunnan 650101, China.
E-mail address: lihuazhang33@sina.com (L. Yang).

https://doi.org/10.1016/j.biopha.2018.03.071
Received 3 November 2017; Received in revised form 12 March 2018; Accepted 12 March 2018

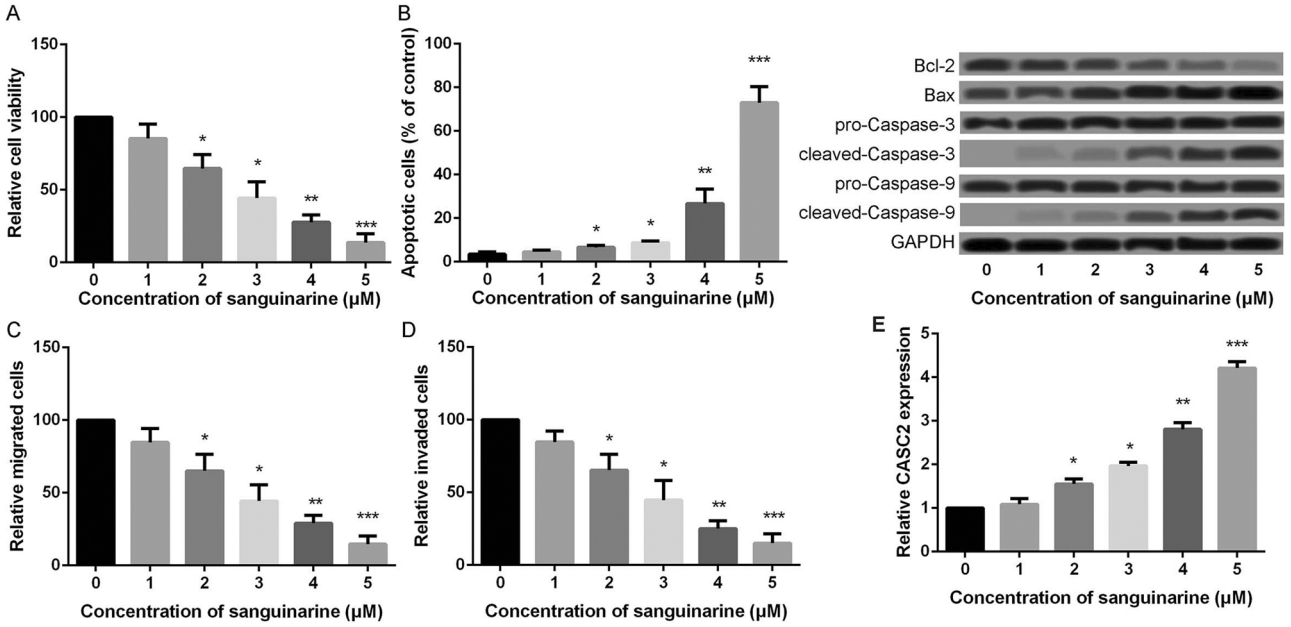

Fig. 1. Effects of sanguinarine on SKOV3 cell viability (A), apoptosis and apoptosis-related proteins (B), migration (C) and invasion (D) after treatment with different concentrations of sanguinarine. (E) The expression of CASC2 in SKOV3 cells after treatment with different concentrations of sanguinarine. All experiments were performed three times independently (n = 3) and the obtained data are expressed as mean ± standard error (SD). *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$ compared to control.

regulators in the development and progression of ovarian cancer, such as HOX transcript antisense RNA (HOTAIR) [14], urothelial cancer associated 1 (UCA1) [15] and human ovarian cancer-specific transcript 2 (HOST2) [16]. Recently, lncRNA cancer susceptibility 2 (CASC2) has been found to plays a crucial roles in variety of cancer, including bladder cancer [17], renal cell carcinoma [18], colorectal cancer [19], non-small cell lung cancer [20] and hepatocellular carcinoma [21]. However, there is a lack of knowledge on the role of lncRNA CASC2 in ovarian cancer.

In the present study, we investigated the effects of sanguinarine on the malignant behaviors of epithelial ovarian cancer cells and the expression of lncRNA CASC2. In addition, we further detected the potential mechanisms of sanguinarine in epithelial ovarian cancer by investigating the interactions between CASC2 and eukaryotic translation initiation factor 4A3 (EIF4A3). Besides, the regulatory relationship between sanguinarine and nuclear factor-κB (NF-κB) signaling or PI3K/AKT/mTOR pathway was explored. All efforts of this study were to lay a theoretical basis for elucidating the pathogenesis of epithelial ovarian cancer and provide new ideas for the treatment of this disease.

2. Materials and methods

2.1. Tissue samples

Twenty patients who were diagnosed as ovarian cancer in our hospital were enrolled in the present study, including 18 patients with epithelial ovarian cancer (13 high-grade serous, 2 low-grade serous, 1 mucinous, 1 endometrioid 1 and 1 clear cell carcinoma) and 2 patients with stromal ovarian cancer. None of the patients had been treated by chemotherapy or radiation therapy before resection of the primary ovarian cancer. The study was approved by the local ethics committee, and informed consent was obtained from all patients. Tumor samples and according normal tissues were immediately frozen in liquid nitrogen and saved at −80 °C.

2.2. Cell culture and treatment

Five human epithelial ovarian cancer cell lines (A2780, Caov3, HO-8910, SKOV3 and OVCAR3) and nonmalignant ovarian epithelial cell line (HOEpiC) were obtained from Chinese Type Culture Collection, Chinese Academy of Sciences. The HOEpiC cells were received at passage 1 and these epithelial ovarian cancer cells were harvested from relatively low passage numbers (< 10). All cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Invitrogen, USA), containing 10% fetal bovine serum, 100 U/ml penicillin sodium, and 100 mg/ml streptomycin sulfate and maintained in a 5% CO_2 humidified atmosphere at 37 °C. The addition of antibiotics was used for inhibiting bacterial growth and avoiding cell contamination. All cells were performed periodic mycoplasma/chlamydia testing every 6 months via short tandem repeat (STR) profiling (IDEXX Laboratories, Columbia, MO).

Sanguinarine was acquired from Sigma chemical co. LTD. (St. Louis, MO, USA). To obtain stock solution, sanguinarine was dissolved in methanol at a 10 mM concentration. The stock solution was stored in aliquots at −20 °C and stabilized for 24 h. The cells were cultured in stock solution with various concentrations of sanguinarine (0, 1, 2, 3, 4 and 5 μM) for another 48 h.

2.3. Cell transfection

Cells were seeded in a 6-well plate and cultured for 24 h before transfection. Human CASC2 gene (NR_026939) was cloned into a pcDNA3.1 (+) vector (Thermo Fisher Scientific, Inc.) to construct pcDNA-CASC2 vector. sh-CASC2, sh-NC, si-EIF4A3 and si-NC were purchased from GenePharma (Shanghai, China). SKOV3 cells were transfected with pcDNA-CASC2 vector or pcDNA empty vector, sh-CASC2, sh-NC, si-EIF4A3 and si-NC using Lipofectamine 2000 (Invitrogen, CA, USA) in accordance with the manufacturer's protocol.

2.4. RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from tissues and cells using Trizol reagent (Invitrogen, CA, USA) in accordance with the manufacturer's protocols. Synthesis of cDNA was obtained from RNA and reverse transcriptase using PrimeScript RT Reagent Kit (Applied Biosystems, CA, USA). The expression levels of CASC2 and EIF4A3 were measured by qRT-PCR in the ABI 7500 system (Applied Biosystems, CA, USA), with GAPDH as a control. The primers were as follows: CASC2, forward 5'-TACAGGAC