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Isoharringtonine inhibits breast cancer stem-like properties and STAT3 signaling

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ABSTRACT

Objectives: Breast cancer stem cells (BCSCs) contribute to breast cancer progression, relapse, and treatment resistance. Identification of the natural inhibitory components of BCSCs is therefore critical for clinical treatment. Here, we investigated whether isoharringtonine (IHT) had inhibitory effects on BCSCs in breast cancer cell lines.

Methods: HCC1806, HCC1937, and MCF7 cells were treated with IHT. The proliferation and the migration of cells were detected by MTS assay and wound healing migration assay, respectively. The proportions of BCSCs were determined by flow cytometry and tumor sphere formation assay. Using real-time quantitative polymerase chain reaction (qRT-PCR) and Western blotting, the expression of Nanog and activation of STAT3 were detected, respectively.

Results: Results showed that IHT inhibited the proliferation of HCC1806, HCC1937, and MCF-7 cells, and suppressed the migration of HCC1806 and HCC1937 cells in a dose-dependent manner. IHT treatment decreased the proportion of BCSCs in MCF-7, HCC1806, and HCC1937 cells. In addition, the mRNA level of Nanong was significantly downregulated after IHT treatment. We also found an inhibitory effect of IHT on STAT3 activation.

Conclusion: IHT inhibited the proliferation, migration, and BCSC proportion of breast cancer cell lines via inhibition of the STAT3/Nanong pathway.

1. Introduction

Breast cancer is a common malignancy and leading cause of cancer-related mortality in women worldwide [1]. Triple-negative breast cancer (TNBC) is a subtype of breast cancer that is loss of estrogen receptor (ER) and progesterone receptor (PR) and lack of over-expression of human epidermal growth factor 2 receptor protein (HER2) [2]. TNBC comprises 15%–20% of all breast cancers [3], and is featured by high recurrence, distant metastasis, and poor overall survival [4]. Due the lack of ER, PR, and specific targets, TNBC is resistant to existing targeted and hormonal treatments [5]. Currently, chemotherapy is still the standard treatment for TNBC, albeit often with limited efficacy and poor survival outcomes [6]. Therefore, TNBC treatment remains a challenge in breast cancer therapy.

Tumor-initiating cells (TICs) are a small population of cells found in the tumor mass, and are often referred to as cancer stem cells (CSCs) due to their properties of self-renewal and differentiation [7]. Growing evidence suggests that CSCs are responsible for tumor initiation, maintenance, heterogeneity, metastatic dissemination, drug resistance, and disease recurrence [8]. Breast cancer stem cells (BCSCs) are defined by higher aldehyde dehydrogenase activity ($ALDH^{high/+}$) and several cell surface markers, including cluster of differentiation 24 and 44 (CD24 and CD44) [9–11]. Transplantation of as few as 1 000 patient-derived $ESA^{+}CD24^{low}CD44^{+}$ cells can reconstitute heterogeneous tumors that are phenotypically similar to the original tumor [10]. These cells are characterized by slow-division, high expression of drug efflux pumps, and high DNA repair ability, which renders them resistant to cancer treatment. Thus, inhibition of BCSCs is a critical treatment strategy for breast cancer, especially TNBC.

Signal transducer and activator of transcription (STAT) proteins are a family consisting of seven transcription factors, including STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 [12]. The JAK/

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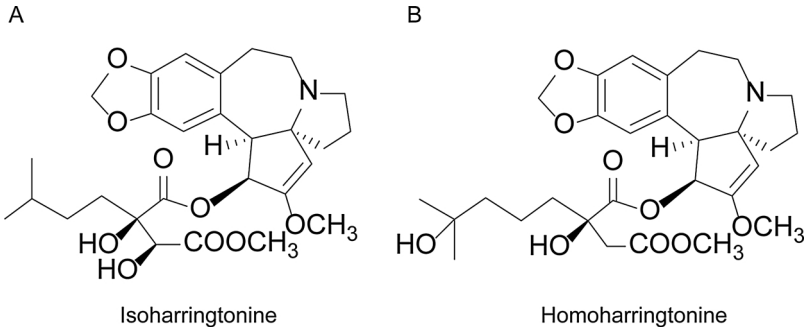

Fig. 1. Chemical structures of IHT and HHT.

STAT signaling pathway acts as a tumor promoter and causes genomic instability, cell cycle dysregulation, and tumorigenicity, which are constitutively activated in many types of cancer cells [13–15]. Within the STAT family, STAT3 also acts as an oncogene and promotes the transcription of Mcl1, p21, Bcl-xl, Bcl-2, c-Myc, and cyclin D1, and therefore contributes to tumorigenesis and tumor development [12]. In addition, activation of STAT3 helps maintain the stem-like properties of cancer cells [7,16]. Thus, the development of STAT3 inhibitors and antagonists are urgently needed for the clinical treatment of BCSCs.

Isoharringtonine (IHT) is a natural analogue of homoharringtonine (HHT), with both compounds extracted from *Cephalotaxus harringtonia* [17]. The chemical structures of IHT and HHT are shown in Fig. 1. Currently, HHT is widely used in clinical treatment for acute myeloid leukemia, myelodysplastic syndrome, acute promyelocytic leukemia, and chronic myeloid leukemia [18]. Although HHT has no effect on advanced colorectal carcinoma, malignant melanoma, sarcoma, and head and neck carcinoma [19,20], recently study has reported that it induces apoptosis and inhibits the IL-6/JAK1/STAT3 signaling pathway in gefitinib-resistant lung cancer cells [12]. These previous studies suggest that HHT may decrease drug resistance in solid cancer cells. Considering the similar structures of HHT and IHT, we supposed that IHT might possess inhibitory effects on BCSCs. To this end, we tested and found that IHT inhibited the proliferation, migration, and BCSC proportion of breast cancer cell lines via inhibition of the STAT3/Nanong pathway. Thus, our findings provide a potential candidate for treatment targeting BCSCs.

2. Materials and methods

2.1. Reagents

Isoharringtonine (IHT ≥ 90%) was extracted from *Cephalotaxus harringtonia* by Professor Xianghai Cai's Lab at the Kunming Institute of Botany, Chinese Academy of Sciences, and was dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the cell culture was below 0.025%.

2.2. Cell culture

The HCC1806 and MCF-7 cells were purchased from the Kunming Cell Bank of the Chinese Academy of Sciences and the HCC1937 cells were purchased from the Shanghai Institute of Biochemistry and Cell Biology, China. The HCC1806 and HCC1937 cells were grown in PRIM 1640 medium supplemented with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin (Gibco, Carlsbad, USA), and cultured in a humidified atmosphere with 5% CO_2 at 37 °C. The MCF-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin, and maintained at 37 °C and 5% CO_2.

2.3. Proliferation assay

Cells were seeded into 96-well plates (2×10^3 cells/well) and precultured for 24 h, with the HCC1806 and MCF-7 cells treated with IHT at concentrations of 0, 50, 100, 200, and 300 nM for 24 h or 48 h, and the HCC1937 cells treated with IHT at concentrations of 0, 300, 400, 500, and 600 nM for 24 h or 48 h. Cell proliferation was then assessed by MTS assay (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. Absorbance was measured at 490 nm with a BioTek Epoch microplate reader (USA). All samples were assayed in triplicate.

2.4. Mammosphere-forming assay

Cells were plated in 96-well ultralow attachment plates at 2×10^3 cells/well. Mammosphere cultures were maintained in MammoCult human medium (STEMCELL Technologies, Vancouver, Canada) supplemented with IHT according to the product's information sheet. For HCC1937 cells, the concentrations of IHT were 0, 400, 500, 600, and 700 nM. For MCF-7 cells, the concentrations of IHT were 0, 50, 100, 200, and 300 nM. After 7 d, mammospheres (sphere-like structures with diameters larger or equal to 100 μm for HCC1937 cells or 50 μm for MCF-7 cells) were clearly detected by optical phase contrast microscopy.

2.5. Wound healing assay

Cell migration capacity was calculated by wound healing assay. We plated HCC1806 and HCC1937 cells into 6-well plates incubated in PRIM 1640 medium with 10% FBS. After reaching 90% confluence, cells were wounded by scraping with a 10-μl pipet tip, followed by thrice washing in PBS and incubation (37 °C, 5% CO_2) in regular medium containing IHT at concentrations of 0, 50, 100, 200, and 300 nM (HCC1806 cells) or 0, 300, 400, 500, and 600 nM (HCC1937 cells). Wounds were observed at 0 h and 16 h (HCC1806 cells) or at 0 h and 12 h (HCC1937 cells). Cell migration distance was calculated by subtracting the wound width at 16 h (HCC1806) or 12 h (HCC1937) from the wound width at 0 h. Three independent assays were conducted.

2.6. RNA isolation and real-time quantitative polymerase chain reaction (qRT-PCR)

After treatment with IHT at the concerntrations as previously for 24 h, total RNA was isolated using TRIzol reagent (Invitrogen, CA, USA) according to the manufacturer's protocols. Reverse transcription was performed with the PrimeScript RT reagent kit (Takara, China) according to the manufacturer's instructions. qRT-PCR was performed using the SYBRSelect Master Mix (Applied Biosystems, USA) on the QuantStudio3 RT-PCR platform (Applied Biosystems, USA) based on the manufacturer's instructions. GAPDH was used as the control to