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Anti-metastatic effect and mechanisms of Wenshen Zhuanggu Formula in human breast cancer cells

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ABSTRACT

Ethnopharmacological relevance: Wenshen Zhuanggu Formula (WSZG), a traditional Chinese medicine (TCM) empirical prescription, has been used to treat the patients with breast cancer bone metastasis as an adjuvant in clinical practice. To explore the anti-metastatic activity and potential mechanisms of WSZG-containing serum (WSZG-CS) on highly bone-metastatic human breast cancer MDA-MB-231BO cells.

Materials and methods: MDA-MB-231BO cells were cultured alone or co-cultured with bone marrow-derived mesenchymal stem cells (BMSCs). Invasion assays were carried out in Matrigel-coated Transwell chambers. CC chemokine 5 (CCL5) and interleukin (IL)-17B secretion levels were detected by ELISA. CCR5 and IL-17BR protein expression levels were determined by immunocytochemistry and Western blot analysis.

Results: Compared with control serum, WSZG-CS significantly inhibited BMSC induced MDA-MB-231BO breast cancer cell invasion, reduced CCL5 and IL-17B levels in co-culture supernatants, and down-regulated CCR5 and IL-17BR protein expression in breast cancer cells co-cultured with BMSCs.

Conclusions: WSZG-CS exerts an anti-metastatic activity against MDA-MB-231BO breast cancer cells, due to its ability to mitigate the interaction between BMSCs and breast cancer cells mediated *via* the CCL5/CCR5 and IL-17B/IL-17BR signaling pathways.

1. Introduction

Bone is the most common distant metastasis site for breast cancer. Over 70% of patients with advanced breast cancer develop bone metastasis, which results in intractable pain, bone fragility, nerve compression, hypercalcaemia, leukoerythroblastic anemia, and eventually death (Mundy, 2002). Patients with breast cancer bone metastasis usually receive systemic treatments, including endocrine therapy, chemotherapy, and/or administration of biological agents. However, these treatments may cause severe side effects and result in reduced tolerability. Herbal medicines as adjuvant treatment are widely used in breast cancer chemoprevention in clinical practice, and their potential mechanisms have been extensively investigated (Liao et al., 2013; Ozer et al., 2000). Some Chinese herbal medicines with bone preserving potential such as Epimedii Folium, Psoraleae Fructus, Cnidii Fructus, and Drynariae Rhizoma have been shown to alleviate cancer pain and prevent bone destruction in animal models of breast cancer bone metastasis (Li et al., 2010; Yao et al., 2012).

Wenshen Zhuanggu Formula (WSZG), a traditional Chinese medicine (TCM) empirical prescription developed in Longhua Hospital, is composed of Psoraleae Fructus (dried matured fruit of *Cullen corylifolium* (L.) Medik., Leguminosae, or Bu-Gu-Zhi in Chinese), Cnidii Fructus (dried matured fruit of *Cnidium monnieri* (L.) Cusson, Apiaceae, or She-Chuang-Zi in Chinese), and Aconiti Lateralis Radix Praeparata (sliced product of the processed lateral or daughter root of *Aconitum carmichaelii* Debeaux, Ranunculaceae, or Fu-Zi in Chinese), and has been used extensively as an adjuvant to treat patients with breast cancer bone metastasis in TCM clinical practice. A retrospective cohort study of 48 patients

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with bone metastases from breast cancer has confirmed that WSZG is effective in ameliorating tumor-induced bone pain, decreasing the incidence of bone-related events, and improving the quality of life (Sun, 2010). Our previous pharmacological study demonstrated that intragastric administration of WSZG significantly reduced the frequency of bone metastasis and mitigated the osteolytic lesions in nude mice with breast cancer bone metastasis (Zhang et al., 2014). In addition, synergism between Psoraleae Fructus and Cnidii Fructus displayed anti-tumor and anti-metastatic actions *in vitro* and *in vivo* by regulating the OPG/RANKL/RANK signaling pathway (Liu et al., 2010; Sheng et al., 2011). However, the mechanisms underlying the anti-metastatic effects of this formula at the cellular and molecular levels are unknown.

Bone marrow-derived mesenchymal stem cells (BMSCs) are multipotent mesenchymal stromal cells, which can differentiate into adipocytes, osteocytes, and chondrocytes (Uccelli et al., 2008). BMSCs facilitate the healing of inflammation sites and injured tissues as tissue regenerative cells and gene delivery vehicles (Prockop, 2009). Increasing evidence suggests that the interaction between BMSCs and breast cancer cells plays an important role in promoting the malignant progression of breast cancer. On the one hand, BMSCs stimulate breast cancer cell survival and growth in the bone marrow through the secretion of chemoattractant cytokines (Fierro et al., 2004; Hombauer and Minguell, 2000). On the other hand, BMSCs are recruited to the sites of primary and metastatic tumors, where they integrate into the tumor microenvironment and contribute to breast cancer cell motility, invasion, and distant metastasis (Bergfeld and DeClerck, 2010; Lazennec and Jorgensen, 2008).

Therefore, BMSC-tumor cell interaction might be considered a potential target for therapeutic intervention in breast cancer metastasis. The present study aimed to explore the anti-metastatic activity and potential mechanisms of WSZG-containing serum by evaluating the effects of this formula on the interaction between BMSCs and MDA-MB-231BO breast cancer cells *in vitro*.

2. Materials and methods

2.1. Plant materials and WSZG preparation

The medicinal materials Psoraleae Fructus, Cnidii Fructus, and Aconiti Lateralis Radix Praeparata were purchased from Shanghai Kang Qiao Chinese Sliced Crude Drug Co. Ltd. (Lot. 12615) and authenticated by Professor Zhi-Li Zhao (Department of Pharmacognosy, Shanghai University of Traditional Chinese Medicine). Morphological, microscopic, and phytochemical identification was performed according to the Pharmacopoeia of the People's Republic of China (2010 edition). The herbarium voucher specimens were deposited in the Institute of Chinese Traditional Surgery, Longhua Hospital affiliated to Shanghai University of Traditional Chinese Medicine, with voucher numbers BGZ-1207081 (Psoraleae Fructus), SCZ-1207082 (Cnidii Fructus), and FZ-1207083 (Aconiti Lateralis Radix Praeparata).

WSZG extract was prepared according to our previous method with minor modifications (Zhang et al., 2014). Briefly, the above three medicinal herbs were mixed at a ratio of 5:5:3 for a total dry weight of 650 g. The herb mixture was immersed in 55% ethanol (1:10 w/v) for 4 h and refluxed for 1.5 h. After filtration, the residue was once more refluxed with 55% ethanol (1:8, w/v) for 1 h and filtered. Then, the two decoctions were combined and concentrated by vacuum to yield a final extract with a concentration of 2 g/mL. Simultaneous quantification of three major active constituents in the extract by high-performance liquid chromatography (HPLC) was employed for quality assurance (Fig. 1). The contents of psoralen, isopsoralen, and osthole in the extract were 15.3, 11.6, and 25.2 mg/g, respectively, which were higher than those in the water WSZG extract (data not shown). Therefore, in this study, 55% ethanol was adopted for WSZG extraction.

2.2. Chemicals and reagents

Human interleukin-17B (IL-17B) and CC chemokine 5 (CCL5) ELISA kits were from Cusabio Biotech Co., Ltd. (Wuhan, China) and Biosource International, Inc. (Camarillo, USA), respectively. Rabbit anti-CCR5 polyclonal and goat anti- IL-17BR antibodies were purchased from Santa Cruz Biotechnology (Shanghai) Co., Ltd. (Shanghai, China). Rabbit anti-goat secondary antibodies were from Jackson Immuno Research Laboratories, Inc. (West Grove, PA, USA). Other chemicals used were of analytical grade and obtained from Sigma (St Louis, MO, USA).

2.3. Preparation of WSZG-containing serum (WSZG-CS)

Male Sprague-Dawley (SD) rats weighing 250–270 g from Shanghai Experimental Animal Center of Chinese Academy of Sciences were housed under 12/12-h dark/light conditions at 22 °C ± 2 °C and relative air humidity of 45–55%, with food and water *ad libitum*. During the study, all animals received humane care in accordance with the *Guide for the Care and Use of Laboratory*

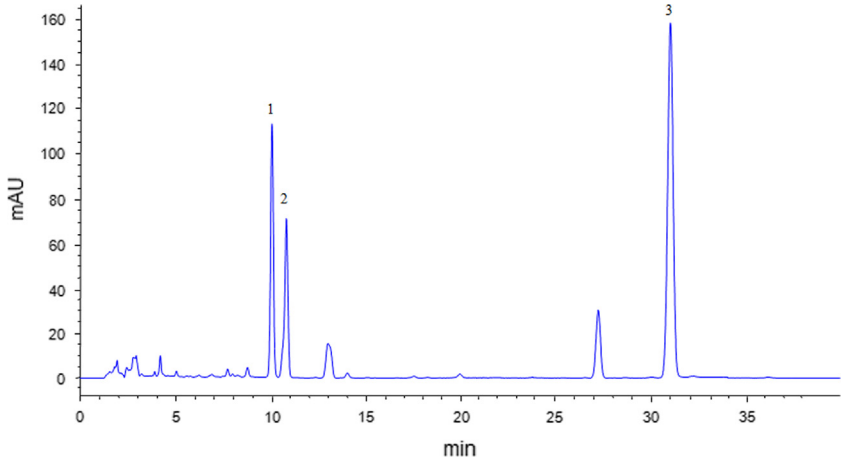

Fig. 1. Chromatographic profile of WSZG extract. 1, psoralen; 2, isopsoralen; 3, osthole. Chromatographic separation was achieved on a Kromasil 100-5 C18 column (250 mm × 4.6 mm, 5 μm) maintained at 25 °C. The mobile phase consisted of acetonitrile (A) and water (B) with a linear gradient elution of 40–50% A (0–20 min), 50–55% A (20–25 min), and 55% A (25–35 min). The flow rate was 1 mL/min and detection was carried out at 325 nm.