



Psoralen inhibits bone metastasis of breast cancer in mice



Chunyu Wu^{a,1}, Zhenping Sun^{a,1}, Yiyi Ye^b, Xianghui Han^b,
Xiaoyun Song^a, Sheng Liu^{a,*}

^a Department of Breast Surgery, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, 725 South Wanping Road, Shanghai 200032, China

^b Pharmacology Laboratory of Traditional Chinese Medicine, Longhua Hospital, Shanghai University of Traditional Chinese Medicine, 725 South Wanping Road, Shanghai 200032, China

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ABSTRACT

Breast cancer is the most common female malignancy and it frequently metastasizes to bone. Metastatic breast cancer continues to be the primary cause of death for women in East and Southeast Asia. Psoralen is a furocoumarin that can be isolated from the seeds of *Psoralea corylifolia* L. Psoralen exhibits a wide range of biological properties and has been demonstrated as an antioxidant, antidepressant, anticancer, antibacterial, and antiviral agent. Additionally, it is involved in the formation and regulation of bone. This study investigated whether psoralen can inhibit metastasis of breast cancer to bone *in vivo*. Histological, molecular biological, and imaging analyses revealed that psoralen inhibits bone metastases in mice. Psoralen may function to inhibit breast cancer cell growth in the bone microenvironment and regulate the function of osteoblasts and osteoclasts in tumor-bearing mice. The results of this study suggest that psoralen is a bone-modifying agent and a potential therapeutic to treat patients with bone metastases.

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1. Introduction

Breast cancer is the most common cancer in Chinese women, with crude rate of 43.19 per 100,000 women in 2007, and it continues to be the primary cause of death for women in East and Southeast Asia [1]. It frequently metastasizes to bone and previous studies report that ~80% of advanced breast cancer patients have developed bone metastases [2]. Patients with bone metastases generally develop bone degeneration, fractures, bone pain, hypercalcemia, and limitations in mobility that severely reduce their quality of life [2].

Psoralea corylifolia L. is a plant commonly used in India and China to treat a range of medical conditions such as asthma, cough, vitiligo, and calvities [3]. *P. corylifolia* L. is composed of a variety of coumarins and flavones, such as psoralen, isopsoralen, and many others [4]. Previous studies have documented a number of therapeutic properties for *P. corylifolia* L. It has been

shown to possess antioxidant [5], antiplatelet [6], antidepressant [7], antidiabetic [8,9], anticancer and immunomodulatory effects [10]. In addition, it has been shown to inhibit DNA polymerase and topoisomerase II [11]. Other studies have demonstrated that *P. corylifolia* L. extracts stimulate proliferation of osteoblasts [12,13] and increase new bone formation in rabbits [14]. Extracts have also been shown to inhibit bone resorption of osteoclasts *in vitro* [15]. We previously reported that a Chinese medicinal formula of *P. corylifolia*–*Cnidium monnieri* could inhibit bone metastasis of breast cancer *in vivo* [16].

Psoralen (Fig. 1) is a coumarin isolated from the seeds of *P. corylifolia* L. and it has been reported to be an antioxidant [17], anticancer [18,19], antifilarial [20], antidepressant [21,22], antiviral [23], and antibacterial [24] agent. Psoralen and its analogs inhibit the growth of multiple human tumor cell types *in vitro*, including breast cancer, non-small cell lung cancer, central nervous system cancer, oral carcinoma, and erythroleukemia cells [18,19,25,26]. We previously reported that psoralen inhibited invasion of the breast cancer cell line MDA-MB-231BO *in vitro* [27]. Psoralen has also been shown to promote new bone formation [28] and

* Corresponding author. Tel.: +86 21 64385700; fax: +86 21 64398310.
E-mail address: sliu_tcm@163.com (S. Liu).

¹ C. Wu and Z. Sun contributed equally to this work.

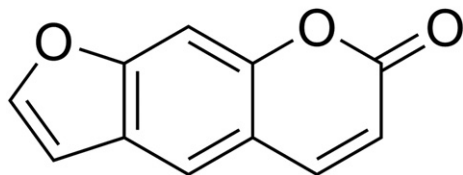


Fig. 1. The molecular structure of psoralen.

stimulate osteoblast differentiation *in vivo* [29,30]. Psoralen could therefore be a candidate therapeutic to inhibit bone metastasis *in vivo*. This study investigated whether psoralen could inhibit metastasis of human breast cancer cells to bone in mice.

2. Materials and methods

2.1. Materials

Psoralen (purity >98%) was purchased from the National Institutes for Food and Drug Control, China. L-15 medium and fetal bovine serum were obtained from Gibco (Grand Island, New York, USA). Antibodies against osteoprotegerin (OPG), receptor activator of nuclear factor kappa-B ligand (RANKL), macrophage colony-stimulating factor (M-CSF), and parathyroid hormone-related peptide (PTHrP) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). An antibody against interleukin-8 (IL-8) was provided by Abcam Technology (Cambridge, MA). Nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) Color Development bone scintigraphy substrate was obtained from Promega Biotech (Madison, Wisconsin, USA). Other chemicals were purchased from Sigma-Aldrich (Saint Louis, Missouri, USA) unless otherwise indicated.

2.2. Animals

Female nude (BALB/c *nu/nu*) mice (20 ± 2.0 g; Shanghai Cancer Institute of Shanghai Jiaotong University; license number 2008-0043 SYXK) were housed in a temperature-controlled (24 ± 2 °C) room with a regular 12-h light/dark cycle. After acclimatization for one week in a specific pathogen-free (SPF) environment, animals were randomly assigned to several experimental groups. All animals had free access to water and food until the day before the experiment. All experiments were performed in accordance with the national regulations for animal experimentation approved by the Shanghai Laboratory Animal Science Administration Commission of Shanghai Municipality.

2.3. Cell lines and cell culture

MDA-231BO cells were a gift from Dr. Toshiyuki Yoneda at Osaka University. Cells were cultured in L-15 medium supplemented with 10% (v/v) fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO₂. Cells were washed three times and placed in sterile phosphate-buffered solution (PBS) shortly before implantation. The subclone of MDA-231BO, an exclusively bone-metastatic cell line, was acquired by repeatedly injecting cancer cells into the left ventricle and isolating tumor cells from bone metastasis lesions.

2.4. Intracardiac injection

Intracardiac injection was performed according to our previous work [31] with slight modifications. Briefly, cells were resuspended at 10^6 cells/mL in PBS. Suspended cells (0.1 mL) were injected into the left ventricle using 29 G needles (Terumo, Tokyo, Japan).

2.5. Radionuclide bone scintigraphy

Radionuclide bone scintigraphy analysis was performed as described previously [31] with slight modification. Bone metastasis was evaluated by *in vivo* imaging with radionuclide bone scintigraphy weekly for 2 weeks after inoculation. Static planar images of the entire skeleton were acquired 5–6 h after tail vein injection of 111 MBq (3 mCi) and 0.1 ml of ^{99m}Tc-MDP (Shanghai Syncor Pharmaceutical, Shanghai, China) using a Siemens multi-single photon emission computed tomography (MultiSPECT) equipped with low-energy, high-resolution collimators. A Macintosh-based computer system running ICON 8.5 (Siemens Medical Systems, Malvern, PA, USA) acquired with a 256×256 matrix, zoom 2.67, and 500–800 kilo-counts per frame. Pinhole images of the regional sites were captured on a GE Hawkeye4 Infinia Functional Imaging Scanner (GE Medical Systems, Inc., Waukesha, USA) with a pinhole collimator and using 1024×1024 matrix, Zoom 1.0. The pinhole insert had been designed and built for obtaining ultra-high-resolution images, with the aperture diameter approximately 1 mm. It was mounted on the collimator housing.

2.6. Radiographic imaging

Conventional radiographs were obtained with a Philips Optimus Bucky Diagnost TS X-ray System (Philips Healthcare, Eindhoven, Netherlands). Bone metastases were determined on radiographs 2–8 weeks after the inoculation of tumor cells. The X-ray tube voltage was fixed at 40 kVp, the current at 2 mA and the exposure time at 3 s.

2.7. Histology

Metastatic lesions bones identified by radionuclide scintigraphy and radiography were dissected, sectioned, and stained with hematoxylin and eosin (H&E). Cancer cells were identified and the percentage of cancer cells per high-power field-of-view ($400\times$ magnification) was calculated. Two pathologists independently observed all histological sections. Sections that the two pathologists generated substantially different results for (>5% discrepancy) were reviewed again until agreements were reached.

2.8. Real-time qPCR

Metastatic bone lesions were harvested and stored in liquid N₂. Frozen tissue (0.2–0.25 g) was homogenized with a pestle and total RNA was extracted using TRIZOL (Promega, Madison, WI) according to the manufacturer's instructions. Real-time PCR was performed as previously described [32]. Primers were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China) and their sequences

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