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Antimetastatic effect of prodigiosin through inhibition of tumor invasion

Jing Zhang, Yaling Shen, Jianwen Liu*, Dongzhi Wei*

Department of molecular & Cellular Pharmacology, State Key Laboratory of Bioreactor Engineering, #365, East China University of Science and Technology, Shanghai 200237, PR China

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Abstract

Prodigiosin, a bacterial metabolite, was reported to have immunosuppressive and anticancer activities. In this study, we investigated novel functions of prodigiosin about anti-metastasis and anti-invasion. Prodigiosin dose-dependently inhibited 95-D cells' migration and invasion according to wound healing assay and the Transwell assay. The inhibitive effect could reach about 50% when cells were treated with 5 μM prodigiosin for 12 h. In animal experiment, intraperitoneal administration of 5 mg kg^{-1} prodigiosin decreased the number of metastatic nodules by 53% and elevated the survival rate of mice about one-fold comparing with control group. Results of cell aggregation and adhesion assay showed that prodigiosin could promote cell aggregation and simultaneously inhibit cell from adhering to extracellular matrix (ECM). In addition, prodigiosin suppressed RhoA gene expression, hence, decreased protein level of RhoA in 95-D cells, according to RT-PCR assay and Western blot assay. Gel zymogram assay revealed that prodigiosin could suppress the activity of matrix metalloproteinase-2 (MMP-2). These results demonstrate that prodigiosin effectively inhibit tumor metastasis in vitro and in vivo. The action mechanisms of prodigiosin are associated with the promotion of cell aggregation and the inhibition of various steps in cell invasive process, which include the inhibition of cell adhesion and mobility in a RhoA-dependent way and the suppression of MMP-2 ability.

Keywords: Prodigiosin; Metastasis; Invasion; MMPs; RhoA

A family of natural red pigments called prodigiosins is synthesized from various bacteria such as *Serratia marcescens* [1]. The members of this family include prodigiosin, cycloprodigiosin hydrochloride (cPrG·HCl), uncedylprodigiosin, metacycloprodigiosin and desmethoxyprodigiosin. Prodigiosin, with methoxypyrrole ring in its structure, has several biological activities such as immunomodulatory, antibacterial, antimycotic and antimalarial activities and so on [2,3]. Recently, lots of studies [4–7] imply that prodigiosin has a massive potential in cancer chemotherapy, which draws increasing public attention. The studies on its anticancer effect mainly focus on inducing apoptosis. It has been reported that prodigiosin could induce apoptosis in various kinds of cancer cells, such as haematopoietic, colorectal and gastric cancer cells [4–6]. However, the inhibitory effects of prodigiosin on metastasis and invasion, and the underlying mechanism have not been elucidated.

Metastasis is one of the major causes of mortality in cancer patients [8]. However, the treatment to metastasis is still far from satisfactory. Lack of effective drugs should be responsible for this worrying phenomenon. So it is critical to find new effective drugs to fight against metastasis. The invasion of tumor cells into adjacent tissue is a crucial event in metastasis. Invasion of tumor cells involves multiple processes that depend on specific cell-to-cell and cell-to-ECM (extracellular matrix) interactions [9]. These interactions are mediated directly by specific adhesion receptors and indirectly by extracellular proteinases that mediate degradation of the ECM [9]. Several reports have indicated that RhoA protein, an important signal molecule, is required for cell adhesion and consequently influences

Abbreviations: ECM, extracellular matrix; MMPs, matrix metalloproteinases; BS, bovine serum; i.p., intraperitoneally; i.v., intravenously; MTT, 3-[4,5-dimethyl-thiazol-2-yl]2,5-diphenyltetrazolium bromide; EC_{50}, half effective concentration; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; RT, reverse transcription

* Corresponding author. Tel.: +86 21 64252044; fax: +86 21 64252044.
E-mail addresses: liujian@ecust.edu.cn (J. Liu), dzhwei@ecust.edu.cn (D. Wei).

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many aspects of cell shape and movement [10–12]. In addition, matrix metalloproteinases (MMPs), a well-known family of zinc-binding enzymes, plays an important role in the process of cleaving ECM components. The expression levels of MMPs are correlated with tumor invasiveness [13].

So far, there has been no report on antimetastatic ability of prodigiosin. Therefore, in the present study, we examined the effect of prodigiosin on metastasis of cancer cells in vitro and in vivo, and further investigated the antimetastatic mechanisms of it with special reference to the process of cell invasion.

1. Materials and methods

1.1. Materials

Prodigiosin was kindly provided by Dr. Yaling Shen (East China University of Science and Technology). Its purity >90%.

1.2. Cells and animals

Human highly metastatic lung carcinoma 95-D cells and the highly metastatic substrain B16BL6 of mouse melanoma B16 cells were obtained from Cell Bank of Chinese Academic of Science and were cultured in RPMI Medium 1640 (GIBCO Industries Inc.) and 10% (v/v) dialyzed heat-inactivated bovine serum (BS) (GIBCO Industries Inc.) at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Male C57BL/6 mice (6 weeks old) were obtained form the Animal Center of Chinese Academic of Science, and maintained on standard chow and water.

1.3. Wound healing assay

For wound healing assay, cells were plated in 24-well microtiter cell culture plates. A plastic pipette tip was drawn across the center of the plate to produce a clean 1 mm-wide wound area after the cells have reached confluence. After a 12 h culturing in RPMI Medium 1640 containing 10% (v/v) BS and different concentrations of prodigiosin, cell movement into the wound area was examined. The migration distances between the leading edge of the migrating cells and the edge of the wound were compared [14]. Migration rate = (migration distances of drug treated cells/migration distances of untreated cells) × 100%.

1.4. Invasion assay

Invasiveness into the reconstituted basement membrane Matrigel® (Becton Dickinson Labware) [15,16] was assayed. Cells were incubated with or without different concentrations of prodigiosin for 12 h, and trypsinized to form single-cell suspension in RPMI Medium 1640 (serum-free), which was added into the upper compartment of a Transwell cell culture chamber. 10% (v/v) BS in culture medium was used as chemo-attractant in the lower chamber. After 8 h of incubation, cells remaining on the upper surface of the membrane were removed with a cotton swab. Cells that invaded through the Matrigel®-precoated membrane filter (Becton Dickinson Labware) were fixed, stained and then counted with a microscope.

1.5. Assay of tumor metastasis in an animal model

For injection into mice, cultured B16BL6 cells were detached with trypsin solution, washed twice with PBS, and diluted to a cell density of 5×10^5 cells mL^{-1} in PBS. A portion (0.2 mL) was inoculated into 6-week-old male C57BL/6 mice via the tail vein. Next day, the drug treatment was started. Animals were divided into five groups of seven animals each. Drugs were dissolved in normal saline containing 0.4% (v/v) Tween 80, and injected intraperitoneally (i.p.) into mice once a day for 2 weeks. Control group was i.p. administered vehicle alone. After 3 weeks mice were sacrificed and their lungs were excised, rinsed, and fixed in Bouin's solution. The total number of visible nodules on the lung surface per mice was counted.

1.6. Cell aggregation assay

The cell aggregation assay was performed essentially as described previously [17]. Briefly, a single-cell suspension was obtained through standard trypsinization procedures. A total of 2×10^5 cells in 1 mL of RPMI Medium 1640 (serum-free) with or without different concentrations of prodigiosin was placed in polystyrene microtubes and shake gently every 5 min for 1 h at 37 °C. At the end glutaraldehyde (at a final concentration of 2% (v/v)) was added to stop the aggregation process. The percentage of aggregated cells was calculated as $(1 - Ne/Nc) \times 100\%$, where Ne is the number of single cells after incubation at 37 °C and Nc is the number of single cells before incubation.

1.7. Cell adhesion assay

95-D cells were pre-treated with or without different concentrations of prodigiosin for 12 h. Then cells were suspended in serum-free RPMI Medium 1640 to form a single-cell suspension, and were seeded into 96-well microtiter cell culture plates that had been precoated with Matrigel® (Becton Dickinson Labware). After a 45 min incubation at 37 °C, the wells were washed three times with PBS to remove non-adherent cells. 10 μM of MTT was added to each well for an additional 4 h. The blue MTT formazan precipitate was dissolved in 100 μL of DMSO and the absorbance at 570 nm was measured on a microplate reader (Bio-Rad Laboratories).