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Antimetastatic effect of prodigiosin through inhibition of tumor invasion

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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

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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).

1.8. Western blot analysis of RhoA

95-D cells (1×10^6) were placed in culture dish (10 cm), and treated with or without different concentrations of prodigiosin for 24 h. Cells were washed with PBS twice and extracted in a lysis buffer [10 mM Tris–HCl (pH 7.5) containing 50 mM NaCl, 50 mM NaF, 10 mM EDTA, 1 mM DTT, 1% (v/v) Triton X-100, 0.1% (m/v) SDS, 1% (m/v) sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride, 5 mM leupeptin, and 10 mg mL^{-1} 1 aprotinin] for 30 min in ice. The lysates were centrifuged to remove insoluble materials, normalized according to their protein content. Then equal amounts of proteins (30 μg) were separated by SDS-polyacrylamide gel electrophoresis and blotted onto a PVDF membrane (Amresco). After blocking for 1 h at room temperature using PBST buffer (PBS buffer plus 0.1% (v/v) Tween-20) containing 3% (m/v) BSA, the filter was then applied sequentially with mouse monoclonal anti-RhoA antibody (diluted 1:500; Santa Cruz Biotechnology), and secondary antibody conjugated horseradish peroxidase (diluted 1:500; Santa Cruz Biotechnology). Then the blots were visualized with enhanced chemiluminescence detection and imagined on KodakTM X-OMAT film.

1.9. RhoA mRNA detection by RT-PCR

95-D cells were administered with prodigiosin similar to the Western blot assay. Total RNA was isolated using TRIzol™ reagent (Promega Corporation) and then reverse-transcribed using reverse transcription system (Promega Corporation). An aliquot (4 μL) of RT product was used for PCR amplication in a total volume of 20 μL. RhoA cDNA (417 bp) was amplified using the sense primer 5′-ACC AGT TCC CAG AGG TGT ATGT-3′ and the antisense primer 5′-TTT GGT CTT TGC TGA ACA CT-3′ and GAPDH was used as the load control. The thermal cycle profile used in this study was (1) denaturing for 30 s at 94 °C, (2) annealing primers for 90 s at 55 °C and (3) extending the primers for 30 s at 72 °C. PCR amplification was performed for 30 cycles and a portion (10 μL) of the PCR mixture was visualized by electrophoresis in 1.2% agarose gel containing 0.2% ethidium bromide. The gel was photographed and then quantitatively measured by scanning densitometry.

1.10. Gel zymogram

Proteins with gelatinolytic activity were identified by electrophoresis in the presence of SDS in 9% polyacrylamide gels containing 0.1% (m/v) gelatin. Equal volume of cell culture medium was obtained from cultured 95-D cells in serum-free RPMI Medium 1640 with or without different concentrations of prodigiosin for 24 h. Cultured media were concentrated two-fold and mixed with Laemmeli's sample buffer in the absence of β-mercaptoethanol, loaded onto gels and electrophoresed. After PAGE, the gel was washed with 2.5% (v/v) Triton X-100 and incubated in the buffer, pH 7.4, containing 50 mM Tris, 10 mM $Cacl_2$, 10 μM $Zncl_2$ for 16 h at 37 °C. Clear bands of gelatinolytic activity were visualized after staining the gel with Coomassie blue. The gel was photographed and then quantitatively measured by scanning densitometry.

1.11. Statistical analysis

Experimental values are expressed as the mean ± S.D. By scientific statistic software *GraphPad Instat* version 2.04, One-way ANOVA assay was used to evaluate the significance of differences between groups with statistical significance considered as $^{*}p < 0.05$, $^{**}p < 0.01$ or $^{***}p < 0.001$.

2. Results

2.1. Effect of prodigiosin on the migration and invasion of 95-D cells in vitro

To evaluate the antimetastatic activity of prodigiosin, we first assessed the inhibitory effect of prodigiosin on the migration and invasion of 95-D cells by the wound healing assay and the Transwell assay. As shown in Fig. 1A, the cellular motility was obviously inhibited in

Table 1
Antimetastatic effect of prodigiosin in vivo

Group	Treatment	Number of lung nodules	Survival rate[a] (%)
1	Control	72.00 ± 16.90[b]	43
2	Prodigiosin (2.5 mg kg^{-1} per day)	54.50 ± 9.90	86
3	Prodigiosin (5 mg kg^{-1} per day)	34.00 ± 13.10***	86
4	Prodigiosin (10 mg kg^{-1} per day)	18.50 ± 5.43***	100
5	Vinblastin (2 mg kg^{-1} per day)	15.75 ± 8.98***	86

Seven C57BL6 mice per group were inoculated i.v. with B16BL6 cells (10^5 cells per mouse) and then injected i.p. with drugs for 2 weeks, and then sacrificed at the end of 3 weeks. Experiments were repeated two times with similar results. The data shown are results of a representative experiment.

[a] The numbers indicated the percentage of surviving mice at the end of the experiment.

[b] Mean ± S.D. for each group of mice.

*** $p < 0.001$ significantly different from control group.

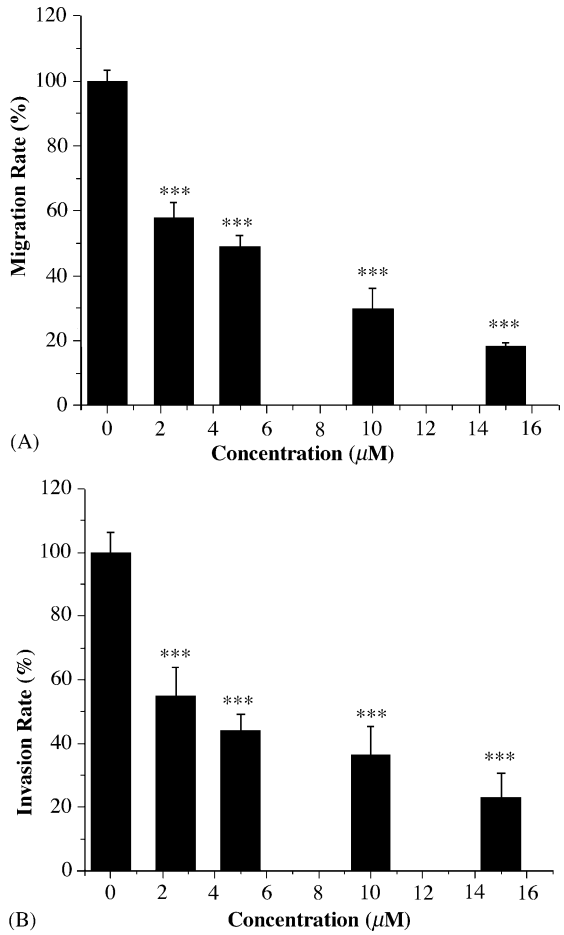

Fig. 1. Effect of prodigiosin on migration and invasion of 95-D cells. (A) Effect of prodigiosin on migration was tested by wound healing assays. A wound was introduced by scraping with a pipette tip when the cells have reached confluence. After 12 h incubation with or without different concentrations of prodigiosin, the migration distances were measured. Migration rate = (migration distances of drug treated cells/migration distances of untreated cells) × 100% [the cell migration distances in various teams in sequence are 1.94, 1.12, 0.95, 0.58, 0.35 mm]. (B) Effect of prodigiosin on invasion. 95-D cells were pretreated with or without different concentrations of prodigiosin for 12 h.Then cells were seeded into the upper compartment of a Transwell cell culture chamber. After 8 h of incubation, the invading cells on the lower surface were dyed and counted. Invasion rate = (the number of invading cells in drug treated team/the number of invading cells in untreated team) × 100% [the number of invading cells in various teams in sequence is 200, 110, 88, 73 and 46]. Experiments were repeated independently three times with similar results. The data shown are results of a representative experiment. All data were presented as the mean ± S.D. of five parallel samples in each team. Statistical significance of differences between untreated and treated cells with prodigiosin was assayed (one-way ANOVA), $^{***}p < 0.001$.

a dose-dependent manner by prodigiosin. Prodigiosin at 2.5 μM decreased the migration of 95-D cell by 42% ($p < 0.001$), and the EC_{50} (half effective concentration means the concentration of the drug when drug's effect reaches 50% compared with control) of prodigiosin was about 4.66 μM. Similarly, the results of the Transwell assay also showed that prodigiosin could inhibit the invasion of 95-D cells in a dose-dependent manner, and by 56% at 5 μM prodigiosin (Fig. 1B). Within the range of dose in experiments, prodigiosin did not exhibit obviously cytotoxicity as determined by the trypan blue exclusion assay (data not shown).

2.2. Effect of prodigiosin on pulmonary metastasis and host survival

Since pulmonary metastasis of mouse melanoma is an acknowledged animal model about metastasis, we choose it to further test the antimetastatic ability of prodigiosin in vivo. For mice that were inoculated with mouse melanoma B16BL6 cells, pulmonary metastatic foci were markedly decreased by intraperitoneally administration with prodigiosin. And as seen in Table 1, this inhibitory effect on pulmonary metastasis was observed in a dose-dependent manner. The inhibitory rate of formation of metastatic nodules reached 53% in the case of administration with 5 mg kg^{-1}prodigiosin. In addition, the survival rate of mice was elevated from 43% (group 1, negative control), to 86% (group 2), and 100% (group 4) at the end. Prodigiosin did not cause body weight loss in mice during the whole treating period (data not shown), which suggests that the treatment of prodigiosin was tolerated well by these experimental animals.

2.3. Effect of prodigiosin on cell aggregation and adhesion

On the base of the data in Fig. 1 and Table 1,we examined action mechanisms of antimetastasis of prodigiosin. Previous studies have demonstrated that cell–cell interactions and cancer cell adhesion to ECM components play important roles in caner metastasis [18]. Fig. 2A showed that prodigiosin promoted spontaneous cell aggregation in a dose-dependent manner. Following the treatment with increasing concentrations of prodigiosin, the cell polymers grew larger. The percentage of aggregated cells was about 50% when cells were treated with 2.5 μM of prodigiosin.

On the other hand, we examined the effects of prodigiosin on 95-D cell adhesion to ECM proteins. Prodigiosin showed an inhibition of Matrigel®-mediated attachment of 95-D cells with the EC_{50} value of 2.5 μM (Fig. 2B).

2.4. Effect of prodigiosin on RhoA expression

RhoA regulates signal transduction pathways that are required for cell adhesion and migration [12]. To determine the effect of prodigiosin on the expression of RhoA protein in 95-D cells, Western blot analysis was carried out. Compared to control, the RhoA protein level in the cells treated with different concentrations of prodigiosin was decreased in a dose-dependant manner (Fig. 3). As assessed by scanning densitometry, the RhoA expression was inhibited by 45% when cells were incubated with 10 μM of prodigiosin for 24 h.

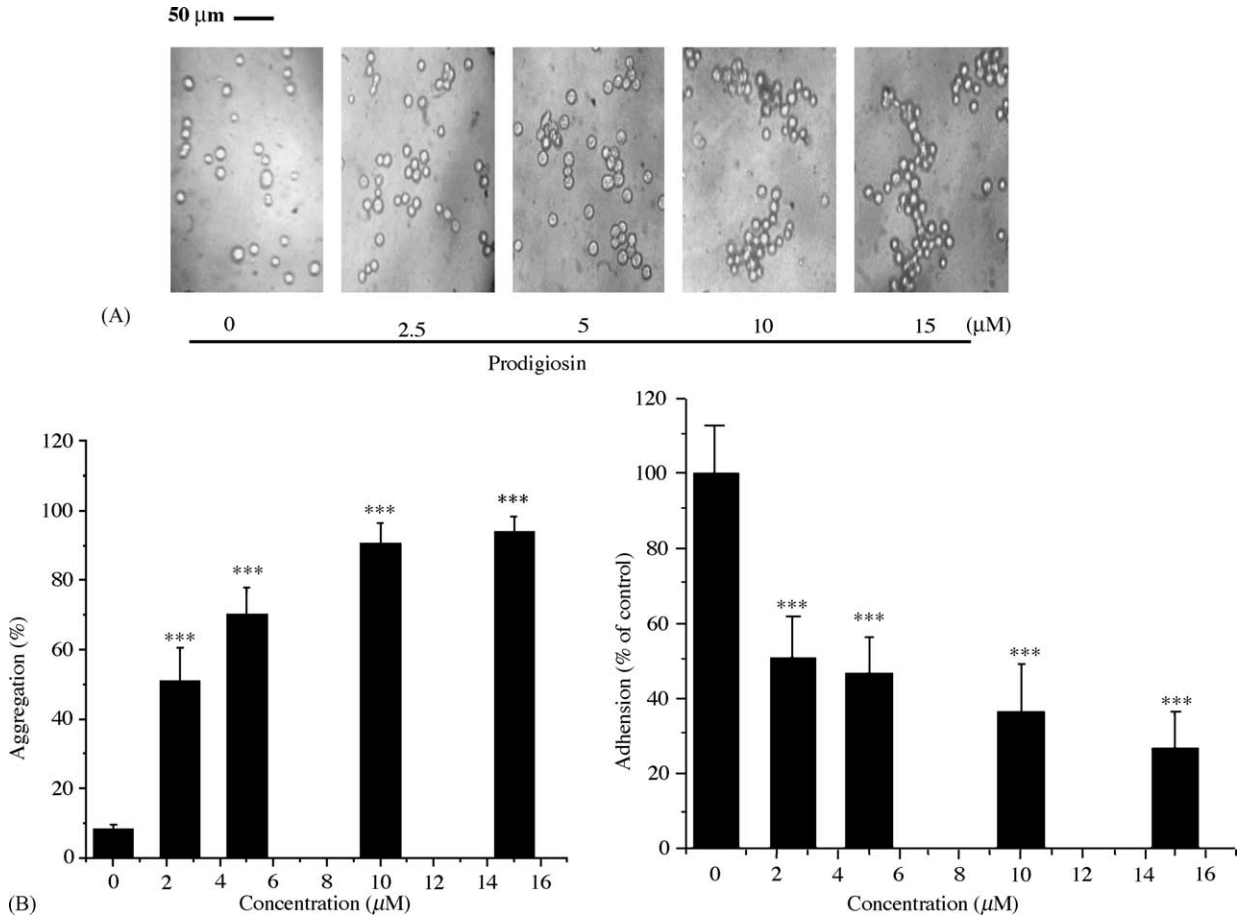

Fig. 2. Effect of prodigiosin on cell aggregation and adhesion. (A) Effect of prodigiosin on cell aggregation. 95-D cell suspensions were treated with serum-free RPMI Medium 1640 containing different concentrations of prodigiosin for 1 h, and then cells were photographed and counted. The percentage of aggregated cells was calculated as $(1 - \text{Ne/Nc}) \times 100\%$, where Ne is the number of single cells after incubation at 37 °C and Nc is the number of single cells before incubation. The scale indicates 50 μm. (B) Effect of prodigiosin on cell adhesion on Matrigel®. 95-D cells were treated with or without different concentrations of prodigiosin for 12 h and placed onto the wells precoated with Matrigel®. After a 45 min incubation at 37 °C, the amount of adhering cells were determined by MTT method. Adhesion rate = (absorbance of drug treated team/absorbance of untreated team) × 100% [the absolute values of absorbance in various teams in sequence are 0.408, 0.207, 0.191, 0.149, and 0.109]. Experiments were repeated independently three times with similar results. The data shown are results of a representative experiment. All data were presented as the mean ± S.D. of five parallel samples in each team. Statistical significance of differences between untreated and treated cells with prodigiosin was assayed (one-way ANOVA), $^{***}p < 0.001$.

We further investigated the effect of prodigiosin on the mRNA level of RhoA. The synthesis of RhoA mRNA in 95-D cells pretreated with prodigiosin for 24 h was also inhibited in a dose-dependent manner, as determined by RT-PCR analysis (Fig. 4). Electrophoresis scanning quantitative analysis indicated that 10 μM prodigiosin could reduce the mRNA level of RhoA by 52%.

2.5. Effect of prodigiosin on degradation of the extracellular matrix

We finally tested the effects of prodigiosin on ECM degradation catalyzed by matrix metalloproteases (MMPs) with gel zymogram. Fig. 5 showed that gelatin zymogram of concentrated serum-free conditioned medium revealed the bands of lysis at 72 kDa (MMP-2). The activity of gelatinase was markedly decreased in secretions from prodigiosin-treated cells, and the subsequent densitometry revealed a 43% inhibition of MMP-2 activity in the cells pretreated with10 μM of prodigiosin.

3. Discussion

Metastasis is one of the important factors related to cancer therapeutic efficacy and prognostic survival [19]. In cancer research, it is one of the most active fields to develop novel antimetastatic drugs with low toxicity and high efficacy. In this study, using wound healing assay and Transwell assay, we found that prodigiosin could effectively inhibit migration of tumor cells in vitro with EC_{50} less than 5 μM (Fig. 1). The formation of metastasis is the result of a series of complex interactions between tumor cells and environment in host [20]. In addition, the environment in host also influences the process of drug getting to its target. So taking the influence of host into account, we

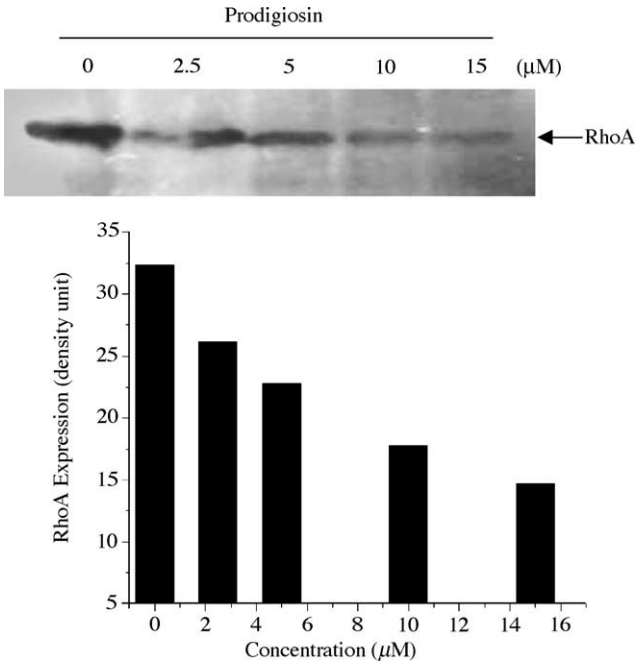

Fig. 3. Effect of prodigiosin on expression of RhoA protein. 95-D cells were treated with or without different concentrations of prodigiosin for 24 h. Equal amount of cellular proteins were separated on SDS-PAGE and blotted with anti-RhoA antibody, and secondary antibody conjugated horseradish peroxidase. Then the blots were visualized with enhanced chemiluminescence detection and imagined on KodakTM X-OMAT film. The film was quantitatively measured by scanning densitometry. Experiments were repeated independently three times with similar results.

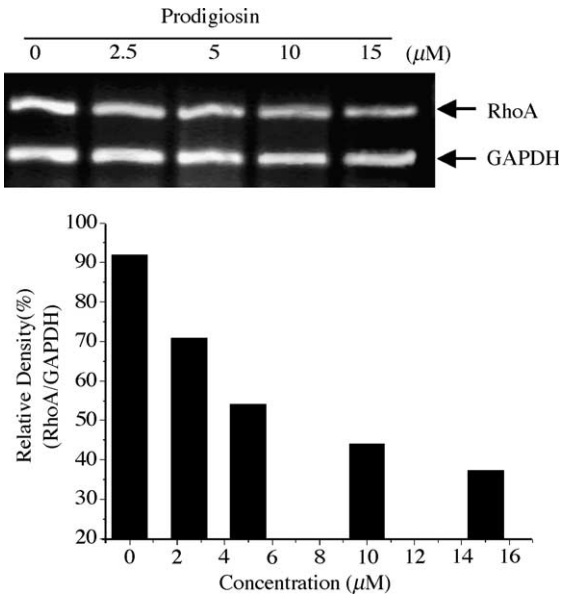

Fig. 4. Effect of prodigiosin on mRNA level of RhoA in 95-D cells. RT-PCR analysis was carried out after the cells were treated with or without different concentrations of prodigiosin for 24 h. After electrophoresis, the gel was photographed and then quantitatively measured by scanning densitometry. GAPDH was used as the load control. Experiments were repeated independently three times with similar results.

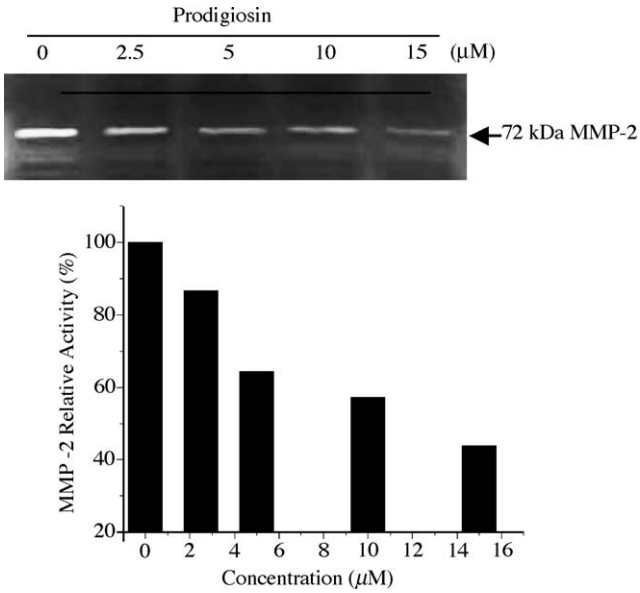

Fig. 5. Effect of prodigiosin on MMP-2 activity. Cells were treated with or without different concentrations of prodigiosin for 24 h in serum-free RPMI Medium 1640. Equal aliquots of media were concentrated and electrophoresed on gelatin gels. The gel was dyed and then quantitatively measured by scanning densitometry. MMP-2 relative activity = (band density of drug treated team/band density of untreated team) × 100% [the absolute values of band density in various teams in sequence are 56.8, 49.2, 36.6, 32.5, and 24.9]. Experiments were repeated independently three times with similar results.

further tested antimetastatic activity of prodigiosin in animal model. In line with the results of cultured cell model, prodigiosin showed the capacity to inhibit cancer metastasis in vivo (Table 1). 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 compared with control group. To our knowledge, it is first reported that prodigiosin has considerable antimetastatic ability in vitro and in vivo. In addition, previous reports indicated that prodigiosin could induce apoptosis in various kinds of cancer cells [4–6]. All these results suggested that prodigiosin should be a promising candidate for cancer chemotherapeutic agent.

Attempting to further understand the action mechanisms of antimetastasis of prodigiosin, we evaluated prodigiosin's effects on cell aggregation and invasion. Metastasis is a multistep process. The initial steps of metastasis include detachment of malignant cells from the primary tumor and invasion into surrounding tissue [21]. In normal tissues, cells are tightly associated with each other, so that they are generally not allowed to migrate freely. However, the malignant cells are more loosely associated, and can freely detach from primary tumor and migrate out [22]. In the present study, we observed that prodigiosin could facilitate cancer cells adhering to each other and forming aggregation (Fig. 2A), which may contribute to prevention of the initial cell release.

The first step of invasion is attachment of cancer cells to ECM components. It has been suggested that adhesive

interactions of tumor cells with ECM components play a critical role in the establishment of metastasis [8,20,23]. In this study, cell adhesion assay showed that prodigiosin could counteract the adhesion of cancer cells to Matrigel®. The adhesive ability of cancer cells was reduced half after cells were pretreated with 2.5 μM prodigiosin (Fig. 2B). The inhibitory effect on cells adhesion might contribute to the inhibition of metastasis by prodigiosin.

The molecular mechanisms by which prodigiosin regulated cell aggregation and adhesion were still unclear. Recently the regulation of cell aggregation, adhesion and migration has become focused on the family of the small Rho-like GTPases, especially RhoA [12,24]. It has been reported that RhoA can regulate signal transduction from cell surface receptors to intracellular target molecules and is involved in a variety of biological processes, including cell morphology, motility, cytokinesis, and tumor progression [25]. Recent evidences suggest that protein expression of RhoA was always higher in primary tumors and lymph node with metastases than in normal tissues [26]. The over-expression of RhoA leads to detachment of cells primary tumors, regulates cell adhesion and promotes tumor cells invasion [10,27]. In the present study, prodigiosin suppressed RhoA gene expression, hence, decreased protein level of RhoA in 95-D cells. RhoA, as an important signal molecule, might play a key role in the signal pathway triggered by prodigiosin. With the results of cell aggregation and adhesion assay, it was possible that through down-regulating RhoA expression, prodigiosin promoted cell aggregation and suppressed cell adhesion to ECM, and then eventually inhibited cell metastasis.

After adhesion, another key step in the invasive progress is the degradation of a variety of ECM proteins by MMPs [28,29]. A central role in this step is played by MMP-2, which cleaves primarily type IV collagen in the basement membrane [28]. Therefore, inhibition of MMP-2 activity is regarded as a rational approach to metastatic disease therapy [28,29]. In this study, it was demonstrated that prodigiosin suppressed the activity of MMP-2 and eventually inhibited cancer cell metastasis. This conclusion is in agreement with those in previous studies that inhibitors of MMP-2 significantly suppressed tumor metastasis in experimental animals [29]. Nevertheless, the underlying molecular mechanisms of this action of prodigiosin remain unknown and require further research in the future.

The invasive process of cancer cells requires that tumor cells first adhere to ECM components, and then secrete MMPs to degrade ECM, and finally migrate through the ECM [30]. We focused on this process and demonstrated that prodigiosin could interfere with multiple steps of invasive progress. It might represent a potential strategy for cancer therapy.

In conclusion, to our knowledge, this study shows for the first time that prodigiosin effectively inhibited tumor metastasis in vitro and in vivo. The antimetastatic effects of prodigiosin were associated with the promotion of cell aggregation and the inhibition of cell invasion, which was evidenced by inhibition of cell adhesion and motility as well as suppression of MMP-2 ability. The down-regulation of RhoA expression may be involved in the possible molecular mechanisms that underlie the anti-invasive effect of prodigiosin. As a new candidate for antimetastatic agent, prodigiosin should be paid more attention, and the further research on the molecular mechanisms of its action will be necessary.

Acknowledgments

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