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Inhibition of β-ionone on SGC-7901 cell proliferation and upregulation of metalloproteinases-1 and -2 expression

Jia-Ren Liu, Bao-Feng Yang, Bing-Qing Chen, Yan-Mei Yang, Hong-Wei Dong, You-Qiang Song

METHODS: Using growth inhibition, Zymograms assays and reverse transcription-polymerase-chain reaction (RT-PCR), we examined cell growth rate activities of matrix metalloproteinases-2 (MMP-2) and -9 (MMP-9), and expression of metalloproteinases-1 (TIMP-1) and -2 (TIMP-2) in SGC-7901 cells after the treatment with β-ionone for 24 h and 48 h, respectively.

RESULTS: β-ionone had an inhibitory effect on the growth of SGC-7901 cells. Eight days after the treatment with β-ionone at concentrations of 25, 50, 100 and 200 µmol/L, the inhibition rates were: 25.9%, 28.2%, 74.4% and 90.1%, respectively. The IC₅₀ value of β-ionone for SGC-7901 cells was estimated to be 89 µmol/L. The effects of β-ionone on MMP-2 and MMP-9 activities in SGC-7901 cells were not observed. However, the levels of TIMP-1 and TIMP-2 transcripts were elevated in cells treated with β-ionone in a dose-dependent manner.

CONCLUSION: β-ionone can inhibit the proliferation of SGC-7901 cells, upregulate the expression of TIMP-1 and TIMP-2 expression, and may influence metastasis of cancer.

http://www.wjgnet.com/v1007-9327/10/167.asp
Zymograms assay for activities of gelatinase
Although many kinds of proteinase are involved in tumor metastasis, members of the matrix metalloproteinase (MMPs, gelatinase) and TIMP families, play a pivotal role in these events. MMPs are a group of zinc-dependent endopeptidase molecules that have the potential to degrade proteins of the extracellular matrix. MMP activity was under regulation at several levels, including activation of the MMP zymogens and specific inhibition by the tissue inhibitors of metalloproteinase (TIMPs)\[54\]. We determined the activities of gelatinase in SGC-7901 cells treated with β-ionone.

The SGC-7901 cells were seeded in 100 ml bottles, each containing 5×10^6 cells. After incubation for 24 h, the medium was replaced with 400 µL of serum-free medium supplemented with β-ionone at different concentrations for 24 h and 48 h. The serum-free media were loaded onto a 100 g/L substrate buffer (50 µg acrylamide gel co-polymerized with 1 g/L gelatin (Amresco Corp, USA) and separated under a non-denaturing condition for 3-4 h. Then, the gels were incubated in 25 g/L Triton X-100 (Sigma, USA) for 1 h and subsequently in a mixture of 0.1 mol/L NaCl, 1 mol/L Tris, pH 7.5, containing 10 mmol/L CaCl_2, 200 mmol/L NaCl and 1 µmol/L ZnCl_2 for 12 to 16 h at 37 °C. Finally, the gels were stained in a solution containing 1 g/L Commassie blue R250, 450 g/L methanol and 100 g/L acetic acid, and the gelatinolytic activity was indicated by bands in a blue background.

### RT-PCR

Tissue inhibitor of TIMP-1 and TIMP-2 were related to several tumorigenic processes in lung, stomach, and mammary gland\[56\]. We examined the expression of TIMP-1 and TIMP-2 in SGC-7901 cells exposed to different concentrations of β-ionone.

SGC-7901 cells (5×10^6) were incubated at different concentrations of β-ionone for 24 h and 48 h, the total cellular RNA was isolated. Concentrations and purity of total RNA were determined. RT-PCR was performed following the manufacturer's instructions (Takara Biotech, Dalian, China). Total RNA (5 µg) and AMV reverse transcriptase XL were used to synthesize cDNA. Twenty-five µL PCR mixture containing 4 µL of RT reaction product, 2.5 U Taq DNA polymerase and 20 pmol primers, was heated for 5 min at 94 °C for pre-denaturation, and then subjected to 35 PCR cycles, denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 45 s, in a PTC-100 thermocycler (MJ Research, USA). TIMP-1 and TIMP-2 genes were amplified with specific primers, with the gene for β-actin as an internal control (Table 1). The amplified products were resolved in a 20 g/L agarose gel containing ethidium bromide, and visualized under ultraviolet light. The density and area of bands were analyzed using the Chemilimage™ 4 000 digital system (Alpha Innotech Corp, USA).

### Table 1 Primer sequences and size of TIMP-1 and TIMP-2 in expected PCR products for RT-PCR

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequences</th>
<th>Product size (bp)</th>
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<tbody>
<tr>
<td>TIMP-1</td>
<td>Sense' 5'-CTGTTGGCTGTGAGGAATGCACAG-3'</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>Antisense' 5'-TCAGAGCCTTTGGAGAAGTGC-3'</td>
<td></td>
</tr>
<tr>
<td>TIMP-2</td>
<td>Sense' 5'-AGACGTAGTGTAGGGCCCA-3'</td>
<td>490</td>
</tr>
<tr>
<td></td>
<td>Antisense' 5'-GTACACAGCGCAGAAAC-3'</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>Sense' 5'-AAGATCTCTCTGTGGGC-3'</td>
<td>532</td>
</tr>
<tr>
<td></td>
<td>Antisense' 5'-CATCCTCGTTCGGAGTGC-3'</td>
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### Statistical analysis

Student’s t test was used for analysis of data. P<0.05 was considered statistically significant.

### RESULTS

#### Cell growth inhibition of β-ionone

The inhibitory effect of β-ionone on the growth of SGC-7901 cells was shown in Figure 1. Growth of the cells in the media containing 200 µmol/L and 100 µmol/L β-ionone was markedly slower than that of the negative control within 8 d (P<0.01). The change was less obvious the cells treated with 50 µmol/L and 25 µmol/L of β-ionone. The inhibitory rates were 25.9%, 28.2%, 74.4% and 90.1%, respectively. The IC50 of β-ionone was 89 µmol/L.

![Figure 1](image1.png)

**Figure 1** Proliferation kinetics of SGC-7901 cells treated at various concentrations of β-ionone.

#### Expression of type IV collagenases

The effects of β-ionone on type IV collagenase activities (97kD for MMP-9 and 72kD for MMP-2) in SGC-7901 cells were presented in Figure 2. In the serum-free supernatants of SGC-7901 cells preincubated in the media supplemented with various concentrations of β-ionone, the activities of type IV collagenases (both MMP-9 and MMP-2) were not different in comparison with the negative control. No effect of β-ionone on the activities of type IV collagenases in SGC-7901 cells was shown.

![Figure 2](image2.png)

**Figure 2** Effect of β-ionone on gelatinase/ type IV collagenase secretion (97kD for MMP-9 and 72kD for MMP-2) in SGC-7901 cells.

#### Levels of TIMP-1 and TIMP-2 mRNA in SGC-7901 cells

As shown in Figures 3 and 4, the levels of TIMP-1 and TIMP-2 transcripts in SGC-7901 cells were gradually elevated with increase of the β-ionone concentration, indicating a upregulation effect of β-ionone on the expression of TIMP-1 and TIMP-2 in SGC-7901 cells.

- **Table 1** Primer sequences and size of TIMP-1 and TIMP-2 in expected PCR products for RT-PCR

- **Figure 1** Proliferation kinetics of SGC-7901 cells treated at various concentrations of β-ionone.

- **Figure 2** Effect of β-ionone on gelatinase/ type IV collagenase secretion (97kD for MMP-9 and 72kD for MMP-2) in SGC-7901 cells.

- **Levels of TIMP-1 and TIMP-2 mRNA in SGC-7901 cells**

As shown in Figures 3 and 4, the levels of TIMP-1 and TIMP-2 transcripts in SGC-7901 cells were gradually elevated with increase of the β-ionone concentration, indicating a upregulation effect of β-ionone on the expression of TIMP-1 and TIMP-2 in SGC-7901 cells.
**DISCUSSION**

Interactions between cells and extracellular matrix (ECM) were critical for many cellular functions including division, migration, differentiation, and apoptosis\[43\]. In different mature normal tissues, ECM is specified by its structures and compositions to maintain tissue homeostasis and cellular quiescence. It has been found that remodeling of the ECM occurred during embryonic development and certain normal pathological processes such as wound-healing\[36,37\]. However, restructuring of the ECM has also been implicated in the pathogenesis of various human diseases including impaired wound-healing and fibrosis\[38\], rheumatoid arthritis\[39\], restenosis following balloon angioplasty\[40\], atherosclerosis\[41\], tumor development, invasion and metastasis\[35,42\].

Tumor invasion is a complex biological process, during which tumor cells detach from the primary tumor and infiltrate the surrounding tissues. This process has been found to require loss of cell contacts between tumor cells, active cell migration, adhesion to ECM and proteolytic degradation of the ECM\[43,44\]. Different molecules including cadherins, integrins, proteases, and growth factors were implicated in the regulation of invasion of tumor cells\[44\].

A major group of enzymes responsible for ECM degradation in cancer tissue has been found to be the MMP family, including collagenases, gelatinase, and stromelysins\[45-49\]. Collagenases can cleave fibrillar collagens at neutral conditions and play an important role in matrix remodeling. The largest sub-family of the MMPs is membrane-type (MT). MT-MMPs play a dual role in cell surface proteolysis. They can cleave a variety of extracellular components, and activate some secreted MMPs. Evidence has suggested that proteolytic activities at cell surface could promote cell invasion\[50\]. MMP-2, a 72-kDa type IV collagenase, is a cell surface-associated type I collagen-degrading MMP, which was found to designate gelatinase A\[51\]. Its overexpression has been observed in different types of tumors, and it was believed to be involved in tumor metastasis, primary tumor growth and angiogenesis\[52\]. MMP-2 is also regarded as type IV collagenase involved in cell invasion across basement membrane. Thus, serum and tissue MMP profiles have been used as prognostic factors in certain types of malignant tumors including gastric and breast cancers\[53\]. MMP-9, a 97-kDa type IV collagenase also designating gelatinase B, was found to be associated with tumor invasion as well, its expression has been proven to be correlated with the differentiation grade of some malignancies\[46\]. In the present study, we did not find any effect of β-ionone on MMP-9 and MMP-2 activities in SGC-7901 cells. The growth inhibition on SGC-7901 cells appeared to be independence of the type IV collagenase activity.

TIMPs (TIMP-1, -2, -3 and -4) have been found to be the key regulators of MMP activity and ECM degradation\[46\]. The MMP inhibitors, TIMP-1 and TIMP-2 have been associated to several tumorigenic processes including development, invasion and metastasis of bronchial cancer\[35-39\]. Recent transgenic animal studies have demonstrated that alteration of the MMP/TIMP balance in vivo in favor of TIMP-1 activity could block neoplastic proliferation in the SV40 T antigen-induced murine hepatocellular carcinoma\[61\]. Active MT1-MMP was found to bind to amino-terminal domain of TIMP-2\[62\], whereas an interaction between the carboxyl-terminal domains of TIMP-2 and pro-MMP-2 was also described\[63\]. TIMP-2 might prevent SH2-protein-tyrosine phosphatase-1 (SHP-1) dissociation from immunoprecipitable epidermal growth factor receptor complex and a selective increase in total SHP-1 activity\[64\]. Our results showed that β-ionone might increase the expression of TIMP-1 and TIMP-2 in SGC-7901 cells. Clearly, further studies are needed to elucidate whether β-ionone inhibits metastasis of the cancer cells.

In summary, β-ionone can inhibit SGC-7901 cell growth
and proliferation and has an effect on upregulating TIMP-1 and TIMP-2 expression in SGC-7901 cells. It seems to be related to potential tumor metastasis in SGC-7901 cells induced by β-ionone. However, the mechanism of the inhibitory effect of β-ionone inhibits cell proliferation remains to be clarified.

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