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Inhibitory effect of chitooligosaccharides on MMP-9 in human fibrosarcoma cells (HT1080)

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Introdution

Chitosan oligosaccharides are depolymerized products of chitosan. Recently, it has reported that chitosan oligosaccharides exhibit a variety of biological functions such as lowering of blood cholesterol, protecting effects against infection, controlling arthritis and tumor growth. In addition, matrix metalloproteinase-9 (MMP-9) has gelatinase activity and playsan important role in cancer invasion and metastasis. Therefore, inhibition of specific types of MMPs including MMP-9 has become an attractive target for therapeutic intervention. In the present reserach, we examined the effect of COS with different molecular weights on human fibrosarcoma cells (HT1080) in order to study MMPs inhibition efficiency of COS.

Meterals and Methods

Gelatin zymography

Cells were exposed to various concentrations of COS with different molecular weights 1 h prior to treatment of 10ng/ml PMA, incubation was continued in FBS-free medium for 3 days. The enzymatic activities of MMPs were determined by zymography as described previously (Hrabec et al., 2001).

RNA extraction

Total RNA was prepared from cells using Trizol (Gibco, USA) according to the manufacturer's instructions. RNA was suspended in DEPC-H2O and stored at -80oC until use. The purity of the RNA was established by reading the optical density of each sample at 260 and 280 nm.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

An aliquot of 1.0 μg of RNA was used for RT-PCR reaction. After that, the PCR reaction was estabilised. Possible DNA contamination was monitored by performing PCR in the same conditions without the addition of cDNA. This procedure was followed so that comparison of gene expression in different samples could be performed under same conditions of amplification. (Zhang et al., 2004)

Genereporter assay

MMP-9 promoter containing reporter vector and beta-gal vector (Promega) were transiently introduced into HT1080 in combination with other indicated constructs using LipofectAMINE/Plus Reagent (Invitrogen). Cells were treated with different concentrations COS for 24 h in media contain serum. After that, cell was extracted and mesure the luciferase activity. (Liao et al., 2003).

Western blot assay

HT1080 cells were treated with different concentrations COS for 24 h in media contain serum. The total protein was extracted and separated using a gradient concentration SDS-polyacrylamide gel/ 5% stacking gel. The resolved proteins were transferred to a nitrocellulose membrane from the gel. Antibody againt Human-MMP-9 was added, followed by addition with anti-mouse serum . The blots were washed again and visualized with enhanced chemiluninescence detection and imagined.

Results and discussion

Our results report that MMP-9 inhibition in the presence of COS was clearly observed in gelatin zymography. Specifically, COS with 1-3 kDa (COS-I), exhibited the highest inhibitory effect on MMP-9 activity in HT1080 cells among tested molecular weight fractions. It was also found that COS-I was capable of inhibiting both gene and protein expression of MMP-9 ($P < 0.01$). In conclusion, COS-I had inhibitory effect on MMP-9 expression and this inhibitory effect was dependent on their molecular weights and concentrations of COS-I. These experimental results in vitro only suggest that COS-I may be used to prevent metastasis involving MMP-9.

References

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