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RESEARCH ARTICLE

ETHANOL EXTRACT OF HERB SAGE SUPPRESSED THE TIMP-1 EXPRESSION IN LYMPHOID CULTURE CELLS

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We aimed to determine the effects of food herbs and spices on several gene expressions. Some herbs and/or spices are found to have a potent transcriptional suppressor activity for the tissue inhibitor of matrix metalloproteinases 1 (TIMP-1) gene. The in vitro effect of the herb Sage ethanol extract on the expression of TIMP-1 was confirmed by reverse transcriptase-polymerase chain reaction (RT-PCR). Western blotting also confirmed the down-regulation of the protein at dose dependent manner of Sage extract in lymphoid Jurkat cell line cells.

Key words: Herb, Spice, gene expression, TIMP-1, Breast cancer, hepatic fibrosis

INTRODUCTION

As herbs have relatively mild bioavailability when taken orally and also low toxicity, the use of herbal intervention is widespread. However, there are insufficient scientific data on the efficacy of herbal therapies. Therefore, basic research aimed at elucidating the mechanisms of action underlying the herbal effects should have high priority. The behavior of a cell is almost dictated by its genetic profile (Kholodenko *et al.,* 2010), investigation of changes in gene expressions as a result of herbal treatment might help define the underlying mechanisms of herbal actions.

TIMP-1, which in turn can promote the deposition of extra cellular matrix, is one of the candidates for prognostic markers in breast cancer, as a number of studies have demonstrated an association between high levels of TIMP-1 and a poor prognosis of breast cancer patients (Schrohl *et al.,* 2006). As proteolytic activity plays a pivotal role in cancer cell invasion and metastasis, the association seems curious, however, recent discovery of other biological functions of TIMP-1 such as growth-stimulating functions, as well as anti-apoptotic and pro-angiogenetic effects, may explain the paradox. Some studies have shown that TIMP-1 is also a very important promoting factor of hepatic fibrosis and inhibits matrix metal protease to deposit extra cellular matrix (Murphy *et al.,* 2004). Strong expression of TIMP-1 reflects the severity of hepatic fibrosis. There seems to be some correlation between the expression levels of TIMP-1 and the severity of hepatic fibrosis. Inhibition of TIMP-1 expression is thus expected to have interference effects on these diseases. It has been reported that traditional Chinese herbs have characteristics of suppressing the expression of TIMP-1 (Lou *et al.,* 2010).

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Therefore, we hypothesized that some other herbs or spices could affect the expression of TIMP-1.

MATERIALS AND METHODS

Cell culture

The human cell lines Jurkat, Daudi and K562 were maintained in RPMI1642 supplemented with 10% fetal bovine serum (FBS), penicillin and streptomycin at 37°C in a humidified atmosphere containing 5% CO2.

Extracts preparation

Herb and spice powders were purchased at food market in Japan. The powders were dissolved in 80% ethanol and subsequently diluted in 40% ethanol at a stock concentration of 50mg/ml. The mixtures were vortexed rigorously for 3 min followed by 3min sonication. After centrifugation (1500 g, 5 min), the supernatants were collected and stored at -20°C until use. For the cell treatments, a range of 0.5-2.0 µl was added to 1 ml of culture medium.

Reverse transcriptase polymerase chain reaction (RT-PCR)

TIMP-1 mRNA was analyzed by semi-quantitative RT-PCR. Total RNA was extracted by RNA isolation Kit (TAKARA, Japan). Two micrograms of total RNA was reverse-transcribed using 1st cDNA synthesis Kit (Clontech) as described in the manufacture's protocol. Cycle based PCR was used to semi-quantitate the TIMP level. GADPH was used as an internal loading control. The primers used for the PCR were designed as follows, TIMP-1 Fw: TGGTAACTCTTTATTTCATTGTCCG, TIMP-1 Rv : CTGAAAAGGGCTTCCAGTCC, (expected size: 183 bp); BRCA1 Fw: AACAGTTTAATTAATACA, BRCA1 Rv : CGGAAATATTTAATAAGTA, (expected size: 166 bp); Leptin Fw : ATGGAATTCGGGAAGGAAAATGCA, Leptin Rv : TCAGTCGACCAGCACCCAGGGCTG, (expected size: 500

bp); GAPDH Fw : TCCCATCACCATCTTCCA, GAPDH Rv : CATCACGCCACAGTTTCC, (expected size: 379 bp).

Western blot analysis

Equal amount of protein samples were used for western blot analysis using anti-TIMP-1 (AnaSpec) and anti-Erk1 (SantaCruz) antibody, and quantified by densitometry. All the western blots were repeated at least three times.

RESULTS AND DISCUSSION

In order to investigate the possibility of using medicinal herb, extracts of some herbs (for example: Rosemary, Green tea, Sage, Kuro-shitimi, Ginger, *Zingiber* mioga and *Perilla* frutescens, etc) were added into cell culture medium of Jurkat or Daudi cells and the levels of TIMP-1 were examined. We employed RT-PCR analysis to quantify the expression level of TIMP-1 gene. Total RNA was isolated 24 hr after extracts treatment for detection of TIMP-1, and the levels of mRNA were determined by the RT-PCR. As shown in Figure 1, the TIMP-1 gene expression level greatly decreased in the treatment of Sage extract at the final concentration 50 □g/ml, compared with the untreated ethanol vehicle group. Expression of the housekeeping gene GAPDH was unaltered. On the contrary, the levels of BRCA1 (Lamber *et al.,* 2010) slightly increased in the treatment of Sage extract (Figure 1). We could not detect the Leptin (Rene Gonzalez *et al.,* 2009) expression in the same condition of this experiment (Figure 1). There was almost no difference on the results of gene expressional profile between Jurkat and Daudi cells. To exclude the possibility of carry-over contamination, reactions containing all RT-PCR reagents including primers without sample RNA were preformed as negative controls. No such RNA contamination was detected (data not shown).

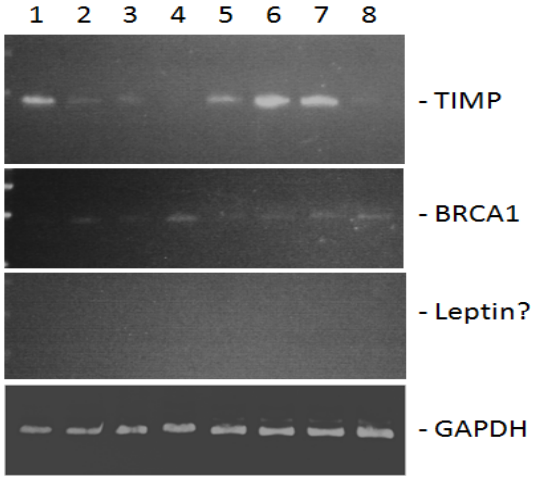

Fig. 1. Semiquantitative RT-PCR was performed using primers specific to TIMP-1, BRCA-1, Leptin, or GAPDH control on 100 ng total RNA prepared from Jurkat cells treated without (lane 1) or with extracts of herbs (lane 2-8: Rosemary, Green tea, Sage, Kuro-shitimi, ginger, *Zingiber* mioga, Perilla frutescens, respectively) at the final concentration 50 μg/ml for 24 hr. Specific expression was determined in relation to the expression of the housekeeping gene GAPDH used as an internal loading control. All the samples were determined within 3 months after collection. At least three independent experiments were done, and typical paired results are documented.

To further confirm the expression status of TIMP-1 induced by the herb extracts, western method was also

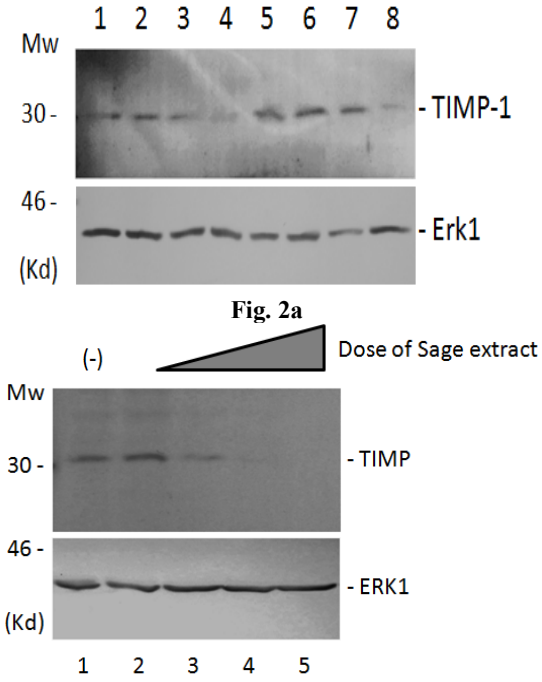

Fig. 2. Sage extract inhibited the expression of TIMP-1 protein. A. Jurkat cells were treated without (lane 1) or with extracts of herbs (lane 2-8: Rosemary, Green tea, Sage, Kuro-shitimi, ginger, *Zingiber* mioga, Perilla frutescens, respectively) at the final concentration 50 μg/ml for 48 hr. After treatment, cell lysates were isolated, the levels of TIMP-1 protein was detected by western blot analysis using anti-TIMP-1 antibody (AnaSpec). Western blot with anti-Erk1antibody (SantaCruz) was also shown as equal levels of protein loading. B. Dose dependent inhibition of TIMP-1 protein expression. Jurkat cells were treated without (lane 1) or with Sage extract at the final concentration 5 μg/ml (lane 2), 25 μg/ml (lane 3), 50 μg/ml (lane 4), 100 μg/ml (lane 5) for 48 hr. The levels of TIMP-1 protein were detected by western blot analysis using anti-TIMP-1 antibody as figure 2A. Western blot with anti-Erk1antibody was also shown.

performed to analyze the level of TIMP-1 protein in the cells. As shown in Figure 2A, the Sage extract also suppressed the protein expression of TIMP-1. This protein expression profile induced by herb extracts approximately agree with the result of RT-PCR shown in Figure 1. We then addressed a question whether the herb can inhibit TIMP-1 expression at dose dependent manner. After pre-treating the cells with a set of different dose of concentrations of the Sage extract, we found that TIMP-1 protein expression was decreased with the increasing concentrations of the extract. Final concentration 100 μg/ml of the Sage extract decreased the TIMP-1 expression by more than 90% (Figure 2B).

Some herbal medications are currently being promoted for a variety of clinical use (Krishnaveni *et al.,* 2010). And herbs have been touted to possess a myriad of beneficial activities, however, limited data and few convincing evidences have been provided at the molecular level. In the results presented here, the expression level of TIMP-1 significantly reduced during treatment with Sage extract, on the contrary, the levels of BRCA1 increased. These

results indicate that Sage potently decreased TIMP-1 expression and offer further support for its potential use in the treatment of breast cancer or hepatic fibrosis (Brocks *et al.,* 2006). To our knowledge, there are no reports on the effects of Sage on TIMP-1 expression. The active components of this herb are unknown. There might be constituents that are critical for its effects. On the other hand, the whole herb could be important of the maximal effect of the agent. The TIMP-1 gene might be complicatedly regulated by various transcription factors. More studies including in vivo experiments need to be undertaken to elucidate the precise molecular mechanisms of this medicinal herb.

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Competing interests statement: The authors declared that no conflict of interest exists.

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