Review: Symposium in the 29th Annual Meeting of Medical and Pharmaceutical Society for WAKAN-YAKU

Basic research on the use of Kampo medicines to protect against cancer recurrence and metastasis

Sumiko Hyuga* and Toshihiko Hanawa

Department of Clinical Research, Oriental Medicine Research Center of Kitasato University, 5-9-1 Shirokanedai, Minato-ku, Tokyo 108-8642, Japan. (Accepted October 30, 2012.)

Abstract

We have focused on the effects of Kampo medicines on cancer metastasis for some time, and 10 years ago we began to assess the medicines in terms of their ability to protect against cancer metastasis. Cancer cell motility is thought to be closely associated with metastatic processes; therefore, we tried to screen Kampo medicines for an inhibitor of cancer cell motility. The samples used for screening were sera from mice given a Kampo medicine such as juzentaihô, hochuekkito, or maoto. The number of migrated cells was significantly reduced in serum obtained from mice given maoto, and the spontaneous metastasis of cancer cells in mice was significantly decreased by the oral administration of maoto. These results suggested that maoto suppresses cancer metastasis through the prevention of cancer cell motility. Furthermore, we investigated the effect of human sera after the intake of maoto on the motility of cancer cells. The sera collected from 10 volunteers after the intake of maoto showed inhibitory activity against cancer cell motility, indicating that maoto may be a novel inhibitor of cancer metastasis suitable for application in humans. To clarify the molecular mechanism by which maoto suppresses cancer cell motility, we focused on the effects of maoto on hepatocyte growth factor (HGF)-c-Met signaling because HGF is one of the growth factors in serum, and it stimulates cell migration through tyrosine phosphorylation of the HGF receptor, c-Met. Maoto prevented HGF-induced cancer cell motility through the inhibition of the phosphorylation of c-Met, and this effect of maoto was derived from its major component, Ephedrae herba. Taken together, these results suggest that maoto may be a candidate drug for protecting patients from the recurrence and metastasis of cancer that expresses c-Met.

Key words cancer, metastasis, maoto, Ephedrae herba, c-Met, HGF.

Abbreviations Akt, a cellular homologue (c-Akt) of the viral oncogene (v-Akt) from the acutely transforming retrovirus AKT8 isolated from a murine T cell lymphoma; CAM, complementary and alternative medicine; ERK, extracellular signal-regulated kinase; FDA, Food and Drug Administration; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HGF, hepatocyte growth factor; HS, human serum; Maotokyomao, Maoto not containing Ephedrae herba; Met, mesenchymal-epithelial transition factor; MS, mouse serum; QOL, quality of life.

Introduction

Cancer patients are typically followed up until recurrence after the completion of cancer therapy; however, there is no therapy that protects against cancer relapse and metastasis. Therefore many cancer patients use complementary and alternative medicine (CAM) containing Kampo medicines to protect against relapse and metastasis. A nationwide survey on the use of CAM in cancer patients in Japan indicated that the prevalence of CAM use was 44.6% in cancer patients.1) The
expected roles that Kampo medicines play in cancer therapy, which include protection against relapse and metastasis, the alleviation of side effects, and the improvement of quality of life (QOL), were described in a guidebook on the use of CAM in the treatment of cancer, which was edited by the study group in Grant-in-Aid for Cancer Research. Thus these reports indicated that Kampo medicines are desired by both cancer patients and clinicians. However, no Kampo medicine has been approved for the treatment of relapse or metastasis in cancer in Japan. We thus undertook basic research aimed at identifying Kampo medicines that can effectively protect against cancer recurrence and metastasis.

**Screening of candidate Kampo medicines for protection against cancer metastasis using our novel assay system**

The recurrence of cancer means that cancer regenerates in the primary organ or that cancer cells metastasize to distant organs and form metastatic lesions after the completion of cancer therapy. We focused on the effects of Kampo medicines on cancer metastasis and screened the medicines for their ability to protect against cancer metastasis using our novel assay system. Cancer cell motility is thought to be closely associated with metastatic processes. Therefore, we tried to screen Kampo medicines for an inhibitor of cancer cell motility. The samples for screening were used sera from mice given a Kampo medicine, as the mode of administration of most Kampo medicines is oral. The motility of the mouse osteosarcoma cell line, highly metastatic FBJ-LL cells, was induced by 0.5% mouse serum. If the serum from a mouse given a Kampo medicine did not induce cancer cell motility, we concluded that the medicine had migration inhibitory activity. We chose three Kampo medicines: juzentaihoto, houhoukikito, and maoto. Juzentaihoto and houhoukikito have been reported to suppress cancer metastasis through activation of the immune system. Maoto was selected as a negative control. However, unexpectedly, the number of migrated cells was significantly reduced by 0.5% sera obtained from mice given maoto (Fig. 1A), whereas there was no significant difference in the number of migrated cells between control serum and serum obtained from mice after the administration of juzentaihoto or houhoukikito. Furthermore, 0.5% normal mouse serum-induced cell motility was also reduced by the addition of maoto (Fig. 1B), suggesting that cancer cell motility is suppressed by the original compound(s) in maoto. Meanwhile, the motility was unchanged by the addition of juzentaihoto.

![Figure 1](image_url) - Effects of mouse serum (MS) obtained from mice given maoto, maoto extract, or its components on FBJ-LL cell motility using the Transwell. (A) The cells (5 x 10⁴ cells) were suspended in 100 µl of RPMI 1640 medium containing 0.5% MS obtained from mice given water (Control), or maoto (A), 100 µl of RPMI 1640 medium containing 0.5% MS with the addition of 0, 0.1, 1, 10, or 100 µg/ml of maoto or juzentaihoto (B), or 100 µl of RPMI 1640 medium containing 0.5% MS with the addition of maoto or its components (C). These cells were poured into the upper well of the Transwell. The lower well of the Transwell contained 600 µl of the same medium in the upper well. At 24 h, cells migrating into the lower well from the upper well were counted. Each assay was performed in triplicate and repeated two times. The error bars represent the standard deviation. Statistical significance was determined by Scheffe’s test (A) or Dunnett’s test (B and C). *p<0.01 in comparison with controls (B and C).
(Fig. 1B). Neither maoto nor juzentaihoto had any effect on FBJ-LL cell growth at 0.1 to 100 μg/ml.3)

The effect of each ingredient in maoto on 0.5% mouse serum-induced motility of FBJ-LL cells was investigated. The inhibitory effect of Ephedrae herba on the motility was as large as that of maoto (Fig. 1C).3) This result suggested that the inhibitory effect of maoto is derived from Ephedrae herba.

Prevention of the spontaneous metastasis of cancer by maoto

To elucidate whether the spontaneous metastasis that arises from a subcutaneous primary tumor was suppressed by oral administration of maoto, we transplanted the FBJ-LL cells subcutaneously into the left thigh of Balb/c mice (5 mice per group) with free access to drinking water or maoto (Fig. 2A). The mice were killed at 4 to 5 weeks. The number of metastatic nodules in the livers in the case of mice given maoto was 1.5 ± 1.3, and that in the case of mice given water was 20 ± 12 (Fig. 2B), indicating that maoto significantly reduced the number of spontaneous liver metastasis of FBJ-LL cells. Meanwhile, juzentaihoto had no effect on the spontaneous metastasis (Fig. 2C). Thus, we found that maoto suppresses cancer metastasis through the prevention of cancer cell motility.10)

Suppression of human cancer cell motility by human serum after intake of maoto

We investigated the effect of human sera collected after the intake of maoto on the motility of human breast cancer MDA-MB-231 cells as a first step in the clinical application of maoto as a metastatic inhibitor. Maoto was orally administered to 10 healthy male volunteers at the usual dosage for 3.5 days (Fig. 3A). Blood samples were collected before the administration of maoto and at

Figure 2 Effects of the oral administration of maoto or juzentaihoto on spontaneous metastasis. Five mice per group were inoculated subcutaneously with FBJ-LL cells (A) and were administered maoto (B) or juzentaihoto (C) with free access to drinking water. After 4-5 weeks, the mice were killed, and the number of liver metastatic nodules was counted. Each bar represents the average of 5 mice ± S.D. The significant difference was determined by Dunnett's test. *p<0.05 vs the mouse given water.
Figure 3 Effect of human serum (HS) after intake of maoto or shikunshito on the motility of human breast cancer cells. (A) Blood samples were collected on the day before intake of maoto or shikunshito. The volunteers (n = 10) took the medicine twice daily orally between meals for 3 days and abstained from food between 21:00 on the 3rd day and 13:00 on the 4th day. On the 4th day, blood samples were collected at 0, 0.5, 1, 2, and 4 hours after oral intake of maoto or shikunshito. (B and C) MDA-MB-231 cells (3 x 10⁶ cells) were suspended in 100 μl of Leibovitz’s L-15 medium containing 1% HS obtained before or after the intake of maoto or shikunshito. To the lower wells 600 μl Leibovitz’s L-15 medium containing 1% HS obtained before or after the intake of the Kampo medicine was added. The Transwell was incubated for 24 h at 37°C, and then the cells migrating to the lower well were counted. Percentage of motility (%) is \((\text{the number of migrated cells with 1% HS obtained at 0-4hr after the intake of Kampo medicines} / \text{the number of migrated cells with 1% HS obtained before the intake of Kampo medicines}) \times 100\%\). “Before” means that human serum obtained before the intake of maoto, and “0 h” means that human serum obtained at 0 hr after the intake of maoto on the 4th day (C). Each assay was performed in triplicate, and the error bars represent the standard deviation. The significant difference was determined by Dunnett’s (B) and Scheffe’s test (C). * p < 0.05 or ** p < 0.01 vs. before intake of Kampo medicines.
0, 0.5, 1, 2, and 4 hours after maoto intake. Shikunshito was also orally administered to 10 healthy male volunteers as a comparison prescription. This study protocol was approved by the ethics committee of the Oriental Medicine Research Center, Kitasato University. The sera from volunteers after the intake of maoto were diluted to 1%, and their inhibitory effects were examined. Figure 3B showed the motility that was induced by the serum from volunteer A, B or C. The motility, which was induced by the serum from volunteers A and B at 30 min after the intake of maoto and the serum from volunteer C at 1 hr after intake, reached a minimum, and the inhibitory ratio of these sera was found to be about 40%. The inhibitory activities of the sera from these volunteers continued for 4 hr after the intake of maoto. The sera from each of the 7 other volunteers showed inhibitory patterns similar to one of the patterns of the serum from volunteer A, B or C. On the other hand, the sera obtained from volunteers after the intake of shikunshito did not inhibit the motility. Figure 3C shows that the sera obtained after the intake of maoto caused nearly 30% reductions in the cancer cell motility.11,12 Although these sera were diluted to 1%, they showed an inhibitory effect on motility. Hence we suspect that the serum obtained from volunteers after the intake of maoto may have a stronger inhibitory activity. These results suggest that maoto may be a novel inhibitor of cancer metastasis suitable for application in humans.

The target molecule of maoto

The molecular mechanism by which maoto suppresses cancer cell motility was not elucidated. We focused on the effects of maoto on hepatocyte growth factor (HGF)-c-Met signaling, because HGF is one of the growth factors in serum, and it stimulates cell migration through tyrosine phosphorylation of c-Met.13,14 The Transwell migration assay demonstrated that maoto prevented the HGF-induced motility of MDA-MB-231 cells, and its major component, Ephedra herba, had the same effect. However, maoto that did not contain Ephedra herba (maotokyomao) had no such effect (Fig. 4A). These results indicated that the inhibitory effect of maoto can be attributed to Ephedra herba. To confirm the effects of maoto and Ephedra herba on HGF-c-Met signaling, we examined the effects of these medicines on the HGF-induced phosphorylation of c-Met. Both maoto and Ephedra herba inhibited c-Met phosphorylation, but maotokyomao had no such effects (Figs. 4B and C). Moreover, Ephedra herba directly inhibited the tyrosine kinase activity of c-Met (Fig. 4D) and suppressed the HGF-induced phosphorylation of Akt, which is a signal molecule downstream of c-Met (Fig. 4E). Taken together, these results suggested that Ephedra herba inhibits the HGF-induced motility of MDA-MB-231 cells via the suppression of HGF-c-Met-Akt signaling through the inhibition of c-Met tyrosine kinase (Fig 4F).

Then, we confirmed the inhibitory effects of maoto and Ephedra herba on HGF-c-Met signaling in other c-Met expressing cancer cells as follows: 1) Scattering of human hepatocellular carcinoma HepG2 cells, which is induced by HGF, was inhibited by maoto or Ephedra herba through the prevention of c-Met phosphorylation15; 2) the growth of human hepatoma HuH-7 cells was stimulated by HGF, but the growth was suppressed by maoto or Ephedra herba. HGF-induced phospho-
Figure 4  Effect of maoto or Ephedrae herba on both HGF-induced motility and tyrosine phosphorylation of c-Met. (A) MDA-MB-231 cells (5 x 10^5 cells) were suspended in DMEM with or without maoto, Ephedrae herba, maotokyoumao, or shikunshito extracts and poured into the upper well of the Transwell. The lower well contained 600 μl of DMEM containing 50 ng/ml HGF. At 20 h, the cells migrating into the lower well were counted. Each assay was performed in triplicate, and the error bars represent the standard deviation. The statistical significance was determined by Dunnett’s test. * P<0.01 vs. the control. (B) MDA-MB-231 cells were incubated in DMEM, DMEM containing HGF, or DMEM containing HGF with maoto, Ephedrae herba, maotokyoumao, or shikunshito for 5 min at 37°C. The level of tyrosine phosphorylation of c-Met in each cell group was then determined by immunoprecipitation and Western blot analysis. (C) The cells were incubated in DMEM containing HGF with 0 to 10 μg/ml Ephedrae herba for 5 min at 37°C. Then the levels of tyrosine phosphorylation of c-Met in the cells were determined. (D) The tyrosine-kinase activity of c-Met was measured using a ProfilerPro Kit. (E) The cells were incubated in DMEM containing HGF with 0 to 10 μg/ml Ephedrae herba for 5 min at 37°C. Then the levels of phosphorylation of Akt and ERK in the cells were determined. (F) Predictive target of Ephedrae herba in HGF-c-Met signal transduction pathway.

Agenda for clinical application of the novel effect-efficacy of Kampo medicines

We cannot apply the novel effect-efficacy of maoto in a clinical setting, because there is no guideline for the application of new botanical drugs in Japan. Meanwhile, the Food and Drug Administration (FDA) in the USA enacted a guideline titled “Guidance for Industry Botanical Drug Products” in 2004. Clinical trials of Kampo medicines such as TU-100(7) and PHY906 are in progress under the guidance of the FDA. TU-100 is daikenchuto, which is prescribed to improve gastrointestinal motility and prevent postoperative adhesion and

riration of c-Met in the cells was also inhibited by them. Furthermore, the growth of tumors formed by HuH-7 cells in vivo was prevented by the oral administration of Ephedrae herba (unpublished data).

These results suggested that maoto and Kampo medicines containing Ephedrae herba are applicable as a novel type of c-Met inhibitor for c-Met-expressing cancer patients. We confirmed that the principal components of Ephedrae herba, ephedrine and its derivatives, have no effect on HGF-c-Met signaling. We are now analyzing the active ingredients in Ephedrae herba.
paralytic ileus after abdominal surgery in Japan.\textsuperscript{15} The evaluation of the effects of TU-100 on gastrointestinal and colonic transit in humans is currently underway in the USA. PHY906 is ongoing which has been used to treat gastrointestinal distress. Recently, preclinical study has indicated that PHY906 enhances the antitumor efficacies of a broad spectrum of anticancer drugs,\textsuperscript{18,19} and the phase I/II studies of PHY906 as a modulator of chemotherapy are in progress in the USA.\textsuperscript{20,21} If these Kampo medicines are approved as new drugs in the USA, they may be introduced in Japan. We think that the guideline for the application of new botanical drugs has to be enacted in Japan before this happens.

**Conclusion**

We showed that maoto is a candidate drug for protecting cancer patients from cancer recurrence and metastasis. We clarified that the inhibitory effects of HGF-c-Met signaling induced by maoto are derived from its major component, Ephedrae herba. Thus, it is possible that Kampo medicines containing Ephedrae herba would be useful for c-Met-expressing cancer patients.

**Acknowledgements**

We thank our collaborators, Dr. Masashi Hyuga, Dr. Goda Yukihiro, Dr. Hidenori Ito, Ms. Masumi Shiraiishi, and Dr. Hayao Nakanishi. We are extremely grateful to Prof. Naoki Inagaki for giving us the chance to write this review.

**References**


