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ORIGINAL ARTICLE

Scabrous patrinia root inhibits circulating tumor cells in differentiated thyroid carcinoma – a clinical observation*

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Abstract

Objective To observe the clinical inhibition of circulating tumor cells (CTCs) in differentiated thyroid carcinoma (DTC) by the extract of scabrous patrinia root (Huikangling).

Methods Eighty-seven DTC patients tested positive for CTCs were randomly divided into two groups; all patients were treated with oral levothyroxine sodium in accordance with the DTC endocrine inhibition treatment criteria. Patients (n = 45) in the treatment group were provided the standard endocrine therapy along with oral Huikangling (0.4 g/tablet, 0.4 g × 3 / time, 3 times / day, 12 weeks). Patients (n = 42) in the control group were only provided the standard therapy. After 4 and 12 weeks, CTCs in the blood were detected by flow cytometry.

Results After 4 weeks of oral Huikangling treatment, CTCs were detected in 18 (40%) and 29 (69%) patients in the treatment and control groups, respectively; the difference was statistically significant (χ^2 = 8.49, P < 0.05). After 12 weeks, CTCs were detected in 7 (15.6%) and 17 (44.7%) patients in the treatment and control groups, respectively; the difference was statistically significant (χ^2 = 5.68, P < 0.05). Follow-up evaluation revealed two patients with lung metastasis and one patient with bone metastasis in the control group; one patient showed lateral neck lymph node metastasis without local recurrence in the treatment group.

Conclusion Huikangling treatment reduces the number of CTC-positive DTC cases; however, further studies are needed to elucidate the underlying mechanisms.

Key words: scabrous patrinia root; Huikangling; differentiated thyroid carcinoma; flow cytometry; circulating tumor cell

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Differentiated thyroid carcinoma (DTC) is characterized by delayed onset and slow progression. Therefore, DTC patients present with different degrees of metastasis at the time of diagnosis; bone, lung, or other distal metastases are also reported to occur in the early phases [1]. DTC is associated with good overall prognosis, and DTC patients display a 10-year survival rate of more than 85%; however, the 10-year survival rate of patients with combined distal metastases plummets between 13% and 21% [2]. Consequently, distal metastasis in DTC hinders current therapy, and its prevention is crucial. The detection and elimination of subclinical metastatic cells reduce the incidences of distal metastasis. Circulating tumor cells (CTCs) were detected in the blood using flow cytometry in patients diagnosed with DTC between February 2008 and November 2013, and CTC-positive patients were treated with tablets containing the refined extract of Patrinia scabra Bunge (Huikangling tablets); a positive treatment outcome was achieved, as reported below.

Materials and methods

Inclusion criteria

Patients meeting the following criteria were included: (1) Clinically diagnosed with DTC and confirmed by histological evaluation [3–4]; (2) Not treated by chemotherapy, radiation therapy, or radionuclide therapy before blood sample collection; (3) Treated

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by surgically operated for primary tumor resection; (4) Tested double positive by flow cytometry for cytokeratin 19 (CK19) and polymorphic epithelial mucin 1 (MUC1) in the peripheral blood [5]; and (5) Agreed to provide their informed consent.

Exclusion criteria

Patients diagnosed with DTC for the second time recurrence and requiring radionuclide treatment.

CTC detection

Abdominal venous blood (5 mL) was collected and 10% EDTA-Na2 was added as an anticoagulant. The anticoagulated whole blood samples were labeled with antibodies against CK19 (eBioscience, USA) and MUC1/CD227 (Abcam, USA). A FACS Calibur flow cytometer (BD, USA) was used for detection; a 15-mW argon ion laser (output power, 300 μW; excitation wavelength, 488 nm) was used as the excitation light source. Forward and side scatter parameters were manipulated to allow accurate detection and amplification of the signal-to-noise ratio. A single-parameter channel-filtered histogram was plotted with logarithmic relative fluorescence intensity on the abscissa and relative cell number (frequency) on the ordinate. CK19- and MUC1-positive cells were calculated as the percentages of the circulating blood cells. Single positive CK19 or MUC1 cells were excluded, and only those cells, which were double positive for CK19 and MUC1, were considered as CTC-positive cases [5].

Clinical background of the patients

Patients included in this study were diagnosed with DTC at the Department of Head and Neck Surgery, Gansu Provincial Cancer Hospital between February 2008 and November 2013; treatment and follow-up evaluations were performed according to the standard protocol based on the tumor stage and pathology [3, 4, 6]. Patients meeting the inclusion criteria (n = 87) were randomly divided into the treatment group (n = 45; 10 males and 35 females; 16–72 years old, median = 38.3 years) and the control group (n = 42; 9 males and 33 females; 16–70 years old, median = 38.1 years). Tumor staging revealed 7 T1, 18 T2, 11 T3, and 9 T4 cases in the treatment group, and 6 T1, 17 T2, 9 T3, and 10 T4 cases in the control group; analysis of lymph node metastasis revealed 15 N0 and 30 N1 cases in the treatment group, and 14 N0 and 28 N1 cases in the control group; pathological classification revealed 33 well-differentiated and 12 intermediately differentiated tumor cases in the treatment group, and 31 well-differentiated and 11 intermediately differentiated tumor cases in the control group.

Treatments

Patients in both the groups were treated with oral levothyroxine sodium (L-T4, 50 μg/tablet), starting from a low dose (25 μg / time, once a day) to a high dose (75 μg, once a day). The doses were gradually adjusted over 1 week according to thyroid stimulating hormone levels, tumor recurrence risk, and side effects of endocrine inhibition as well as in accordance with DTC endocrine therapy standards [6]. In addition, patients (n = 45) in the treatment group were orally administered Huikangling tablets containing the refined extract of scabrous patrinia root, prepared at the Gansu Provincial Cancer Hospital (Z04010883; 0.4 g/tablet, 3 tablets each time, 3 times a day, for 12 weeks). Patients (n = 42) in the control group did not receive any other treatment apart from the standard endocrine therapy. One week after surgery, and in the 4th and 12th week of oral Huikangling treatment, 5 ml of abdominal venous blood was collected, mixed with 10% EDTA-Na_2, and analyzed for CTCs within 24 h.

Observation of treatment outcomes and statistical analysis

After 4 and 12 weeks of starting oral Huikangling treatment, blood samples were tested for CTC positivity; these results were combined with follow-up data for further comparison and analysis. Adverse reactions were assessed according to uniform standards [7]. All the data were analyzed by the SPSS 17.0 software. Intergroup differences were analyzed by Fisher's exact test or χ^2 analysis, and the differences were considered statistically significant when $P < 0.05$.

Results

Number of CTC-positive patients

After 4 weeks of treatment with Huikangling, 18 out of 45 patients in the treatment group and 29 out of 42 patients in the control group were CTC-positive; the difference between the two groups was statistically significant ($\chi^2 = 5.68$, $P = 0.017$). After 12 weeks, 7 patients in the treatment group and 17 patients in the control group were CTC-positive; the difference between the two groups was statistically significant ($\chi^2 = 8.49$, $P = 0.003$).

Follow-up reports

All patients were followed-up for 0.5–7 years (median = 4.7 years). Upon follow-up evaluation, two cases of lung metastasis and one case of bone metastasis were observed in the control group, whereas one case of lateral neck lymph node metastasis without local recurrence was observed in the treatment group. No cases of mortality were observed.

Adverse reactions

Nausea and dizziness were reported by the patients in the treatment group; however, they were abolished after providing symptomatic treatment. No cases of liver and kidney dysfunction were reported.

Discussion

Despite its slow progression, DTC has a good prognosis. CTCs are mainly responsible for its metastasis, which is the main cause of death. Therefore, early intervention is important for the treatment and prognosis. Tumor invasion and metastasis can occur at an early stage of tumorigenesis. Subclinical metastases are observed in approximately 26.3% of the patients at the time of DTC diagnosis [8]. CTCs have recently attracted wide attention because they play a major role in the process of tumor metastasis. They are valuable in the clinical detection of tumor metastases through a procedure known as liquid biopsy [9–10]. CK19 is a low-molecular-weight keratin that is expressed in epithelial, but not in mesenchymal cells, including blood cells. Therefore, the detection of CK19-positive cells in the peripheral blood indicates the presence of epithelial cells in the blood, indicating metastasis of epithelial tumors. Consequently, CK19-positive cells in the blood is used as a clinical marker of tumor metastasis [11]. MUC1 is an epithelial tissue-specific marker, which encodes for a core mucin protein on epithelial cell membranes. Normally, it is not expressed in the peripheral blood, bone marrow, and lymph nodes; therefore, its presence indicates CTCs [12–13]. Both CK19 and MUC1 are expressed only by epithelial tumor cells and are rarely detected in non-epithelial cells. In this study, we exploited their ability to detect CTCs in peripheral blood samples of DTC patients as an indicator of tumor metastasis [14].

Individualized drug therapy targeting CTCs is still in its nascent stages in the field of cancer therapy. The roots of Patrinia scabra Bunge and Patrinia heterophylla Bunge [15] are commonly known as scabrous patrinia roots. P. scabra has immunomodulatory, anti-tumor, sedative, anti-inflammatory, and anti-leukemic activities [16–17]. We used Huikangling tablets, prepared from the refined extract of the scabrous patrinia root, to reduce the number of CTCs in the blood of DTC patients. The number of CTC-positive cases in the treatment group significantly differed from that in the control group after 4 ($P < 0.05$) and 12 ($P < 0.01$) weeks of treatment. The number of CTC-positive cases decreased with increase in treatment duration (12 weeks), which indicated that Huikangling had an inhibitory effect on peripheral blood CTCs; however, further studies are required to understand its mechanism of action.

An extract of P. scabra regulates cell-mediated immunity, changes the immune status of the body, and increases tumor cell detection by the immune cells [18]. It also promotes the cytotoxicity of natural killer cells and the phagocytic activity of macrophages, and improves immune function, thereby exerting anti-tumor effects [19–20]. Chemotherapy significantly alters CTC levels in the peripheral blood; however, residual CTCs lead to poor cancer prognosis [21]. Despite 12 weeks of treatment with Huikangling, seven CTC-positive cases, including five cases of intermediately-differentiated carcinoma, were observed. Intermediately-differentiated carcinoma cells are poorly differentiated, and are capable of rapid proliferation and metastasis; these cells easily diffuse into the peripheral circulation. Such patients often present with distal metastases. Thus, it is crucial to detect CTCs in real-time to analyze the efficacy of treatment and cancer prognosis.

Despite an increase in the number of reports on individualized treatment for DTC patients, the importance of CTCs in these patients has not been emphasized. The number of CTC-negative cases in the control group was 21 (55.3%) after 12 weeks of treatment with Huikangling; however, the 17 CTC-positive patients (44.7%) were at a high risk of developing distal metastases. This risk continually increases with time [22]. Follow-up reports showed two cases of lung metastasis and one case of bone metastasis in the control group, and no cases of distal metastasis in the treatment group. This suggests that the detection of CTCs allows accurate prediction of the risk of distal metastasis; therefore, early intervention and real-time monitoring for CTCs in the blood have great significance. The presence of CTCs in the blood after the radical resection of DTC nullifies any benefit due to the surgical intervention because of a high chance of tumor recurrence. Therefore, the early inhibition of CTCs and the promotion of DTC differentiation serve to reduce distal metastasis.

Conflicts of interest

The authors indicated no potential conflicts of interest.

References

1. Hay ID, Gonzalez-Losada T, Reinalda MS, *et al*. Long-term outcome in 215 children and adolescents with papillary thyroid cancer treated during 1940 through 2008. World J Surg, 2010, 34: 1192–1202.
2. Huang IC, Chou FF, Liu RT, *et al*. Long-term outcomes of distant metastasis from differentiated thyroid carcinoma. Clin Endocrinol (Oxf), 2012, 76: 439–447.
3. Tang ZY. Modern Oncology. Third edition. Shanghai: Fudan University Press, 2011.1370–1383.
4. Ji XL, Ji M. Pathological diagnosis of the thyroid. Beijing: People's military medical press, 2011. 183–256.
5. Ni YQ, Liu QJ, Tian YX. Clinical value of cancer cells joint detection in peripheral blood plasma of thyroid cancer patients. Chinese-German

J Clin Oncol, 2014, 13: 518–522.
6. Chinese Medical Association endocrine credits, the Chinese Anti-cancer Association and head and neck cancer professional committee, the Chinese Medical Association Surgical Branch of the endocrine group, *et al*. Thyroid nodules and differentiated thyroid cancer diagnosis and treatment guidelines. Chin J Clin Oncol (Chinese), 2012, 39: 1249–1272.
7. Gao WJ, Liu XY, Yuan CR. International evaluation system for adverse events of chemotherapeutic drugs in cancer treatment: CTCAE v4.0. Tumor, 2012, 32: 142–144.
8. Nixon IJ, Ganly I, Palmer FL, *et al*. Disease-related death in patients who were considered free of macroscopic disease after initial treatment of well-differentiated thyroid carcinoma. Thyroid, 2011, 21: 501–504.
9. Lianidou ES, Markou A, Strati A. Molecular characterization of circulating tumor cells in breast cancer: challenges and promises for individualized cancer treatment. Cancer Metastasis Rev, 2012, 31: 663–671.
10. Bidard FC, Peeters DJ, Fehm T, *et al*. Clinical validity of circulating tumour cells in patients with metastatic breast cancer: apooled analysis of individual patient data. Lancet Oncol, 2014, 15: 406–414.
11. Tunca B, Egeli U, Cecener G, *et al*. CK19, CK20, EGFR and HER2 status of circulating tumor cells in patients with breast cancer. Tumori, 2012, 98: 243–251.
12. Nath S, Mukherjee P. MUC1: a multifaceted oncoprotein with a key role in cancer progression. Trends Mol Med, 2014, 22: 31–38.
13. Morari EC, Silva JR, Guilhen AC, *et al*. Muc-1 expression may help characterize thyroid nodules but does not predict patients' outcome. Endocr Pathol, 2010, 21: 242–249.
14. Wei WT, Liu QJ, Yao W. Factors influencing the presence of circulating differentiated thyroid cancer cells in the thyroidectomy perioperative period. Oncol Transl Med, 2015, 1: 208–211.
15. National Traditional Chinese Medicine Administration "Chinese Materia Medica" editorial board. Chinese Materia Medica 60. Shanghai: Shanghai Science and Technology Press, 1999. 567–570.
16. Lu GL, Wang YQ, Chen J, *et al*. Advances in chemical constituents and pharmacological effects of patrinia. Chin Arch Tradit Chin Med (Chinese), 2011, 29: 1801–1803.
17. Ma QH, Shi XF, Fan B, *et al*. Research progress on chemical constituents and pharmacological activities of patrinia scabra bunge. Chin Pharm, 2010, 13: 879–881.
18. Wang XX, Zhao JX, Chen WD, *et al*. Effects of patrinia scabra bunge macroporous adsorptive resins extracts on erythrocyte immune function in tumor bearing mice. Chin J Integr Tradit Western Med (Chinese), 2007, 27: 732–735.
19. Chu ZY, Li TJ, Qiu Y, *et al*. Effects of iridoid glycosides on the immune function of mice in Patrinia scabra Bunge. J Chin Med Mater (Chinese), 2008, 31: 882–884.
20. Wang XX, Lu L, Wang M, *et al*. Effect of patrinia scabra bunge macroporous adsorption resin extract on the immune function of S180 tumor – bearing mice. Pharm Clin Chin Mater Med (Chinese), 2012, 28: 71–74.
21. Yin JJ, Zhou LK, Li HL, *et al*. Meta-analysis of circulating tumor cells in breast cancer patients. Chin J Clin Oncol (Chinese), 2012, 39: 602–606.
22. Akagi Y, Kinugasa T, Adachi Y, *et al*. Prognostic significance of isolated tumor cells in patients with colorectal cancer in recent 10-year studies. Mol Clin Oncol, 2013, 1: 582–592.

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