# Herbal complex suppresses telomerase activity in chemo-endocrine resistant cancer cell lines

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## Summary

A herbal complex consisting of Hoelen, Angelicae radix, Scutellariae radix and Glycyrrhizae radix suppressed cell viability and telomerase activity in hormone-refractory and chemo-resistant cancer cell lines, namely poorly differentiated uterine endometrial cancer cell line AN3 CA, adriamycin-resistant breast cancer cell line MCF7/ADR and cisplatin-resistant ovarian cancer cell line A2780. Furthermore, the herbal complex suppressed the expression of the full length of human telomerase reverse transcriptase (hTERT), which is related to telomerase activity. This indicates that the herbal complex can suppress the tumor growth of chemo-endocrine resistant cancers, at least in part via suppression of telomerase activity associated with down-regulated hTERT.

*Key words:* Herbal complex (Hoelen, Angelicae Radix, Scutellariae Radix and Glycycrrhizae Radix); Telomerase activity; AN3 CA; MCF7/ADR; A2780.

## Introduction

Chemo-endocrine therapy has been used in advanced uterine endometrial, breast and ovarian cancers. However, hormone refractoriness and chemo-resistance have been induced after long-term chemo-endocrine therapy in such cancers. Scutellariae radix up-regulates systemic immunity and inhibits the cytotoxicity of anti-cancer drugs, resulting in modulation of the growth of cancer cells [1]. A polysaccharide, AR-4 in Angelicae radix, showed interferon-inducing activity [2]. Furthermore, AR-4E-2 showed potent antitumor activity against the ascitic form of Sarcoma-180, IMC carcinoma and Meth A fibrosarcoma as well as the solid form of MM-46 tumor [3]. Dimeric ellagitaninis showed antitumor activity against Sarcoma-180 cells inoculated to the peritoneum in mice [4]. Glycyrrhetin in Glycyrrhizae radix suppresses the induction of tumor promoters for skin tumor formation [5].

Telomerase is activated in immortalized cancer cells apart from normal somatic cells [6]. Human telomerase reverse transcriptase (hTERT), hEST2 [7] and hTCS1 [8] enzyme catalytic subunits are strongly associated with telomerase activity in cancer cells [9]. In the cancers and cancer cell lines of the female genital tract, telomerase activity is modulated by hTERT mRNA expression [10, 11]. Furthermore, telomerase activity is not regulated only by hTERT, but also by alternate splicing of hTERT transcripts. Excluding three kinds of alternate splicing patterns, hTERT α-deletion (421bp), β-deletion (257bp), and α, β-deletion transcripts (239bp), a full-length hTERT transcript (457bp) coding an active reverse transcriptase might indicate telomerase activity [12]. The intensity of telomerase activity has been a target in cancer therapy [13].

This background prompted us to study whether the complex of the herbal extracts Hoelen, Angelicae radix, Scutellariae radix and Glycyrrhizae radix, which are common components of the Chinese herbal medicines Go-rin-san and Sei-hai-to and also demonstrated to be the main active components of Sho-saiko-to and Juzen-taiho-to [14, 15], can suppress the cell viability of chemo-endocrine resistant cancer cell lines via a telomerase-related pathway.

## Materials and Methods

*Herbal Complex*

The herbal complex consisted of Hoelen, Angelicae radix, Scutellariae radix and Glycyrrhizae radix, the ratio of dried weight being 3:2:2:1. The complex was obtained from Tong Ren Tang (Beijing, China). The complex was boiled for 30 minutes for extraction. The extract was evaporated, concentrated and dried to make the herbal complex powder. The powder dissolved in conventional medium (Eagle's minimum essential medium with 10% fetal bovine serum) was used for each experiment.

*Cancer cell lines*

Hormone-refractory AN3 CA cells derived from poorly-differentiated endometrial cancer, adriamycin-resistant MCF7 (MCF7/ADR) cells derived from breast cancer and cisplatin-resistant A2780 cells derived from ovarian cancer, cultured in the conventional medium, were used.

*Cell viability analysis by the 3-(4,5-dimethyl thizol-2-yl)-2,5-diphenyl terazolium bromide (MTT) assay [16]*

The cells ($1 \times 10^4$) seeded in 3 cm in diameter wells were treated with each concentration (10, 3.3, 1.0 and 0.33 mg/ml) of the herb complex in the conventional medium for 48 hours. After washing the cells with phosphate buffer (PBS), the cells in each well were incubated in 10 μl of 5 mg/ml MTT dissolved in PBS for 4 hours. Then 100 μl of the dissolving solution (34 μl of conc. HCl with 9.966 ml isopropanol) was added to the cells in each well. Absorbance of the dissolved solution was measured using the Easy Reader EAR400 (SLT-Labin Stru-

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ments, Austria) at 550 nm and 620 nm. The absorbance without the treatment was designated as 100%.

*Telomeric repeat amplification protocol (TRAP) assay*

TRAP assay was performed using a TRAPeze telomerase detection Kit (Oncor, Gaithersburg, MD, USA). The cell extract (2 μl) was prepared from $10^3$ cells after treatment with the herb complex. The subsequent protocol was followed from the kit instructions.

*hTERT transcript expression analysis*

Total RNA was isolated from the cancer cells after treatment with the herb complex using ISOGEN (Nippon Gene, Tokyo, Japan). The total RNA (3 μg) was reversed transcribed using a First-Strand cDNA Synthesis Kit (Pharmacia, Uppsala, Sweden). The expression of the reverse transcriptase domain of hTERT mRNA was analyzed using a nested PCR. The first PCR (consisting of denaturing at 94°C for 1 min, annealing at 37°C for 1 min, and extension at 72°C for 2 mins) for the hTERT mRNA with reverse transcribed cDNAs, 2.5 mM deoxynucleotide phosphate mixture, and Taq DNA polymerase (Takara Biochemicals, Ohtsu, Japan) with its buffer for 30 cycles using F-S and F-AS primers as follows: F-S:5'-GTCTCACCTCGAGGGTGAAG-3'; F-AS: 5'CTGATGGAGGTCCGGGCATAG-3' was carried out. To exclude non-specific PCR products from the hTERT mRNA, after incubation of the PCR product at 96°C for 3 mins, the second PCR (consisting of denaturing at 94°C for 1 min, annealing at 62°C for 1 min as a strict condition, and extension at 72°C for 1 min 30 sec) for the hTERT mRNA with the first PCR product and the same solution for 30 cycles using S-S and S-AS primers as follows: S-S: 5'-GCCTGAGCTGTACTTTGTCAAGGACA-3; S-AS: 5'-CGCAAACAGCTTGTTCTCCATGTC-3' was carried out. The expression of human glyceraldehyde-3-phophate dehydrogenase (GAPDH) mRNA as an internal control was analyzed using PCR (consisting of denaturing at 94°C for 50 sec, annealing at 55°C for 50 sec, and extension at 72°C for 90 sec) with the reverse transcribed cDNAs for 31 cycles using GAPDH-S and GAPDH-AS primers as follows: GAPDH-S: 5'-TGAAGGTCGGAGTCAACG-3'; GAPDH-AS: 5'-CATGTGGGCCATGAGGTCCACCAC-3'. The PCR products were analyzed by electrophoresis using 2% agarose gel.

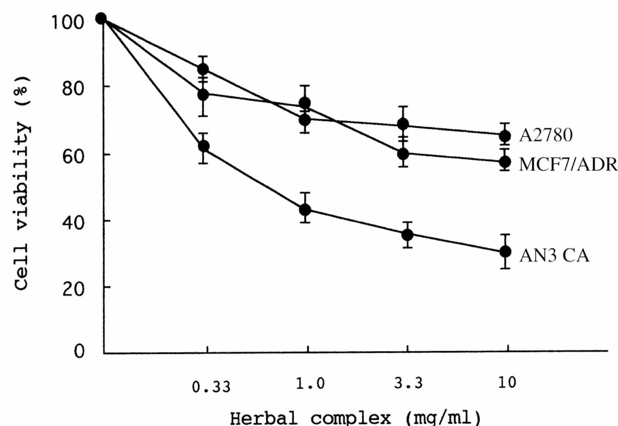


Figure 1. — The effects of the herbal complex on cell viability of cancer cell lines.
The cell viability of AN3 CA, MCF7 ADR and A2780 cells was determined by the MTT assay after a 48-hr treatment with the herbal complex. The value of the cell viability is the mean ± SD of five determinations.

## Results

The cell viability of AN3 CA, MCF-7/ADR and A2780 cells was suppressed dose-dependently at 0.33, 1.0, 3.3 and 10 mg/ml by the herbal complex, especially that of AN3 CA, as shown in Figure 1.

Telomerase activity could be found as a ladder in the band at 6-base intervals beginning at 50 bp. The band of the internal standard was 37 bp. The telomerase activities of AN3 CA, MCF-7/ADR and A2780 cells were suppressed dose-dependently at 0.33, 1.0, 3.3 and 10 mg/ml by the herbal complex as shown in Figure 2.

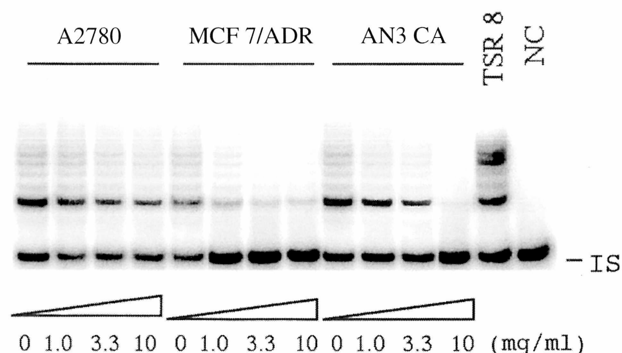


Figure 2. — The effects of the herbal complex on telomerase activity of cancer cell lines.
Telomerase activity of the cancer cells was determined by the TRAP assay. TSR8: telomerase quantification control template, NC: negative control, IS: internal standard. Data representative of the five experiments are shown.

In AN3 CA and A2780 cells, the expressions of full-length hTERT transcript (457 bp), and two alternate splicing α-deletion (421 bp) and β-deletion transcripts (257 bp) were clearly detected. In MCF-7/ADR, the expressions of full-length hTERT and α-deletion transcripts were clearly detected. The expressions of the full-length hTERT transcript of AN3 CA, MCF-7/ADR and A2780 cells were suppressed dose-dependently as shown in Figure 3.

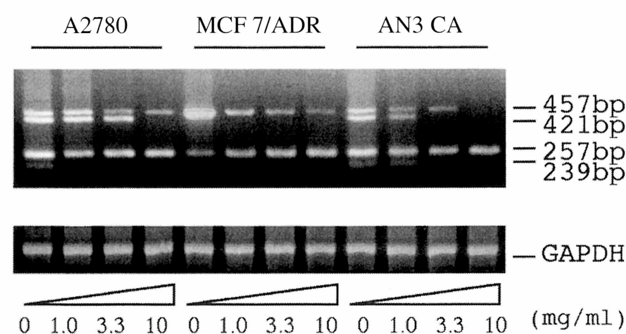


Figure 3. — The effects of the herbal complex on hTERT mRNA expression of cancer cell lines.
The molecular sizes for full-length hTERT (457 bp), α-deletion (421 bp), β-deletion (257 bp), and α, β-deletion transcripts (239 bp) are shown. The expression of GAPDH mRNA is shown as an internal standard. Data representative of the five experiments are shown.

## Discussion

After long-term treatment with chemo-endocrine therapy in advanced uterine endometrial, breast and ovarian cancers, the cancer cells may transform to obtain hormone refractoriness and chemo-resistance. We have confirmed that the herbal complex consisting of Hoelen, Angelicae radix, Scutellariae radix and Glycyrrhizae radix can suppress cell viability and telomerase activity of hormone refractory and chemo-resistant cancer cells. Furthermore, we confirmed the suppression of the full length of hTERT in the cancer cells, which might be associated with the suppression of telomerase activity. Generally, cancer cells conserve telomerase activity to avoid programmed death [6]. Suppression of telomerase activity of the cancer cells might lead to programmed death with apoptosis of the cancer cells [17], and thus the suppression of telomerase activity might influence, at least in part, cell viability reduction. Therefore, we believe that the herbal complex can suppress the tumor growth of chemo-endocrine resistant cancers at least in part via suppression of telomerase activity associated with down-regulated hTERT. Clinically, the herbal complex can be used to avoid the much more severe side-effects of chemotherapy and radiotherapy, and alternatively treat advanced chemo-endocrine resistant cancers.

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