Preliminary report

Monitoring of immune responses to a herbal immuno-modulator in patients with advanced colorectal cancer

Xiao Chen a,1, Ze-Ping Hu b,1, Xiao-Xia Yang b, Min Huang c,*, Yihuai Gao d, Wenbo Tang e, Sui Yung Chan b, Xihu Dai f, Jinxian Ye g, Paul Chi-Liu Ho b, Wei Duan h, Hong-Yuan Yang h, Yi-Zhun Zhi i, Shu-Feng Zhou b,*

a Department of Clinical Pharmacy, 1st Affiliated Hospital, Sun Yat-sen University, Guangzhou, China
b Department of Pharmacy, Faculty of Science, National University of Singapore, Singapore
c Institute of Clinical Pharmacology, School of Pharmaceutical Science, Sun Yat-sen University, Guangzhou, China
d Institute of Food, Nutrition and Human Health, Massey University, Auckland, New Zealand
e Division of Traditional Chinese Medicine, New Zealand Institute of Natural Medicines, Auckland, New Zealand
f Department of Internal Medicine, Fuzhou General Hospital of Nanjing Military Region of the Peoples’ Liberation Army, Fuzhou, China
g Department of Integrated Medicine, Affiliated Hospital of Fujian University of Traditional Chinese Medicine, Fuzhou, China
h Department of Biochemistry, Faculty of Medicine, National University of Singapore, Singapore
i Department of Pharmacology, Faculty of Medicine, National University of Singapore, Singapore

Received 6 July 2005; received in revised form 10 August 2005; accepted 30 August 2005

Abstract

Many herbal medicines are widely used as immuno-modulators in Asian countries. *Ganoderma lucidum* (Lingzhi) is one of the most commonly used herbs in Asia and preclinical studies have established that the polysaccharide fractions of *G. lucidum* have potent immuno-modulating effects. However, clinical evidence for this is scanty. The present open-labeled study aimed to evaluate the effects of *G. lucidum* polysaccharides on selected immune functions in patients with advanced colorectal cancer. Forty-seven patients were enrolled and treated with oral *G. lucidum* at 5.4 g/day for 12 weeks. Selected immune parameters were monitored using various immunological methods throughout the study. In 41 assessable cancer patients, treatment with *G. lucidum* tended to increase mitogenic reactivity to phytohemagglutinin, counts of CD3, CD4, CD8 and CD56 lymphocytes, plasma concentrations of interleukin (IL)-2, IL-6 and interferon (IFN)-γ, and NK activity, whereas plasma concentrations of IL-1 and tumor necrosis factor (TNF)-α were decreased. For all of these parameters, no statistical significance was observed when a comparison was conducted between baseline and those values after a 12-week treatment with *G. lucidum*. The changes of IL-1 were correlated with those for IL-6, IFN-γ, CD3, CD4, CD8 and NK activity (p <0.05) and IL-2 changes were correlated with those for IL-6, CD8 and NK activity. The results indicate that *G. lucidum* may have potential immuno-modulating effect in...
patients with advanced colorectal cancer. Further studies are needed to explore the benefits and safety of *G. lucidum* in cancer patients.

© 2005 Elsevier B.V. All rights reserved.

**Keywords: Ganoderma lucidum; Cancer; Polysaccharide; Immune functions; ELISA**

1. **Introduction**

Cancer is now becoming a leading killer for human beings. The major modalities of cancer treatment to date are surgery, radiation, chemotherapy and immunotherapy [1,2]. Conventional chemotherapy aims to kill or disable tumor cells by direct or indirect mechanisms, while preserving the normal cells in the body [3]. However, these therapies are only successful when the cancer is detected at an early stage or limited to certain types of cancer (e.g. leukemia). Due to limited diagnostic means for detecting pre-carcinoma status and cancers at early stages, most patients present in the advanced stage of cancer or with extensive local infiltration. For advanced tumors, in particular those tumors developed from epithelial tissues such as lung, colon, breast, prostate and pancreas, these therapies are less successful. Chemotherapy may fail due to drug resistance and dose-limiting toxicities [2]. Therefore, alternative therapeutic approaches are needed for the management of cancer patients such as colorectal cancer.

The approach to treat advanced cancer using natural medicines has drawn much attention recently [4]. Indeed, some natural medicines have been investigated as anti-cancer agents in cancer patients and some encouraging findings have been observed, although objective responses have rarely been found. Such natural medicines have been reported to serve as biological response modifiers by activating, increasing, and/or restoring the reactivity of immunological effector mechanisms that are involved in resistance to tumor growth and metastasis [5,6].

*Ganoderma lucidum*, a highly ranked medicinal mushroom, has been widely used by Asian people in the belief that it has potent enhancing effect on immune system and anticancer activity. Preclinical studies demonstrated its antitumor activity and further studies indicated that the polysaccharide (PS) fractions were the major active components for the antitumor action [7,8]. PS from *G. lucidum* was found to activate macrophages, T lymphocytes, and NK cells and to induce the production of cytokines such as tumor necrosis factor (TNF-α), interleukins (ILs) and interferons (IFNs) in in vitro with human immune cells and in vivo in mice [8–11]. A recent study in healthy volunteers indicated that oral treatment of *G. lucidum* supplement at 1.44 g/day for 4 weeks had no effect on immune functions [12]. However, data in patients are scanty. This study aimed to investigate the effects of the water-soluble polysaccharide fractions extracted from *G. lucidum* on selected immune functions in patients with advanced colorectal cancer.

2. **Materials and methods**

2.1. **Patient selection**

A non-randomized open clinical trial was conducted to investigate the effect of water-soluble *G. lucidum* polysaccharides on the immune functions in advanced colorectal cancer patients. Patients were recruited into the study if they met the following eligibility criteria: 1) advanced-stage (staged III to IV) colorectal cancer confirmed by endoscopic and pathological examination, 2) ≥4 weeks of interval between prior chemotherapy or radiatherapy and entry, 3) a Karnofsky performance status [13] score ≥60, 4) an expected lifespan of at least 12 weeks, 5) age ≥18 years; and 6) adequate bone marrow function, renal function and liver function. Informed consent was required from patients, following Helsinki–Tokyo Declaration. Ethical approval was from the Institutional Research Ethics Committee. Patients were excluded if they had severe concurrent conditions such as cardiovascular and liver diseases or had taken or were taking any *Ganoderma* preparations or any potential immuno-modulating agents including general tonics. Pregnant or lactating women were also excluded. Off-study criteria were (1) the patient’s desire to withdraw, (2) non-compliance, (3) unusual or unacceptable toxicity, or (4) emerging evidence that *G. lucidum* was of no benefit to patients with a similar tumor type.

As shown in Table 1, 47 patients with advanced colorectal cancer were included in this study. Of these patients, 43 (91.5%) had ≥2 disease/organ sites affected and 45 (95.7%) were treated with various modules including chemotherapy (mainly fluorouracil–leucovorin and irinotecan) and radiation and complementary medicine. Forty-one patients were assessible for immune functions after a 12-week treatment. Six patients were not assessed due to non-compliance (*n* = 2), lost to follow up (*n* = 3) or death (*n* = 1). The death of one patient by week 10 was due to liver failure with metastasis. This patient stopped taking *G. lucidum* by week 7. There was no evidence indicating that his death was due to the side effect of *G. lucidum*.
Table 1
Demographic profiles of study patients with advanced colorectal cancer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patient (n)</td>
<td>47</td>
</tr>
<tr>
<td>Age (years) (mean ± SD)</td>
<td>48.4 ± 7.0 (30–68)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
</tr>
<tr>
<td>No. of smoker</td>
<td>30 (63.8%)</td>
</tr>
<tr>
<td>Original tumor site</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>42 (89.4%)</td>
</tr>
<tr>
<td>Rectum</td>
<td>5 (10.6%)</td>
</tr>
<tr>
<td>No. of patients with ≥2 disease/organ sites affected</td>
<td>43 (91.5%)</td>
</tr>
<tr>
<td>No. of patients with previous chemotherapy/radiation</td>
<td>45 (95.7%)</td>
</tr>
<tr>
<td>No. of patients with surgical resection</td>
<td>35 (74.5%)</td>
</tr>
<tr>
<td>Patients not assessable after</td>
<td></td>
</tr>
<tr>
<td>12-week treatment</td>
<td></td>
</tr>
<tr>
<td>Non-compliance</td>
<td>2</td>
</tr>
<tr>
<td>Lost to follow up</td>
<td>3</td>
</tr>
<tr>
<td>Death</td>
<td>1</td>
</tr>
</tbody>
</table>

2.2. Extraction and characterization of the G. lucidum polysaccharides

The fruiting bodies of G. lucidum collected from southern China were washed, disintegrated and extracted twice with hot water at 70°C for 3 h as described previously [14]. All hot-water extracts were pooled and the polysaccharide-enriched fractions were precipitated by the addition of 75% (v/v) ethanol. The polysaccharide-enriched fraction was further purified by high-performance anion-exchange and gel filtration chromatography using a 1.6 × 105 cm column packed with Sephadex G-25 (Pharmacia, Uppsala, Sweden). The final polysaccharide products (Ganopoly) had an average molecular size of 4.85 × 105 Da, as determined by gel filtration chromatography and the phenol–sulfuric acid method [15]. They consisted of glucose (61.2%), xylose (15.5%), fructose (14.4%), galactose (4.8%) and rhamnose (4.1%) linked together mainly by β-(1→3) glycosidic linkages. The concentration of proteins was 0.35% as determined using the bicinchoninic acid method [16,17]. Triterpenes were not detected in the final extracts by silica gel thin layer chromatography or visualized by UV light shadowing. There was no detectable level of endotoxin (lipopolysaccharide, LPS) in the extracted polysaccharide fractions by using chromogenic limulus amebocyte lysate assay [18,19].

2.3. Drug administration

The extracted G. lucidum polysaccharides were the only therapeutic agent administered during a 12-week study period. Patients were treated with 1800 mg three times daily orally before meals for 12 weeks. Each capsule of the compound contained 600 mg crude extract of G. lucidum, with 25% (w/w) crude polysaccharides. As the fruiting body of G. lucidum contains approximately 0.5% (w/w) polysaccharides, a capsule was equivalent to 9.0 g fruiting body of G. lucidum or a total dose of G. lucidum per day (5400 mg) was equivalent to 81 g fruiting body. The common dose of G. lucidum for folk use in China is 25–100 g per day, depending on the type and severity of diseases [20]. As most herbs used for chronic diseases are administered for at least 1–4 months, we chose 12 weeks as the treatment regimen. G. lucidum was extracted and the drug preparations were manufactured to the GMP standards and provided by Encore International Co., Auckland, New Zealand.

2.4. Cell culture

Human peripheral mononuclear cells were grown in RPMI1640 medium containing 10% (v/v) fetal bovine serum, 100 units/ml of penicillin, and 100 μg/ml of streptomycin (all from In vitro Life Technologies) in a water vapour-saturated atmosphere of 5% CO2 at 37°C.

2.5. Cytotoxicity

The effect of G. lucidum extracts on the growth of human peripheral mononuclear cells was investigated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously [21]. Briefly, a 200 ml (1 × 10^5 cells/ml) volume of an exponentially growing cell suspension was seeded into a 96-well plate (Falcon Co.) and 20 μl of test compound over 0.05–1.0 mg/ml was added. After incubation for 72 h at 37°C, 20 μl of MTT solution (5 mg/ml in phosphate buffered saline, PBS) was added to each well and the plates were incubated for 4 h further at 37°C. The medium was then aspirated carefully from each well and 180 μl of DMSO is added to each well to dissolve the formazan crystals. Optical density was measured at 490 nm with an absorbance reader (Tecan Instruments Inc.). Each experiment was performed in 6–9 replicate wells for each drug concentration and carried out independently 3 times. The IC50 value was defined as the concentration needed for a 50% reduction in the absorbance calculated based on the survival curves.

2.6. Determination of plasma cytokine concentrations

Plasma from patients before and after treatment was assayed for the determination of various cytokines including IL-1, IL-2, IL-6, IFN-γ, and TNF-α using commercially available ELISA kits (R and D Systems, Abingdon, UK). All determinations were performed at least in triplicate.

2.7. Phytohemagglutinin (PHA)-stimulated lymphocyte proliferation

PHA-stimulated lymphocyte proliferation assay was performed using [3H]-thymidine incorporation method as described previously [22]. Mononuclear cells were separated...
from the heparinized blood of cancer patients by Ficoll–Hypaque gradients and maintained in RPMI 1640 medium. An aliquot of the harvested cells (5 × 10⁶ cells/well) was seeded using a 96-well plate in 0.2 ml of RPMI 1640/5% human AB serum and incubated with 10 mg/L of PHA (Sigma-Aldrich). Cells were incubated at 37 °C for 96 h and added with 1 μCi/well of [³H]-thymidine for the last 16 h and the incorporated radioactivity was measured by a γ-counter. All assays were carried out at least in triplicate.

2.8. Flow cytometry

The lymphocyte subset analysis included CD3 (T lymphocyte), CD4 (T-helper cells), CD8 (T-suppressor cells), and CD56 (NK cells). Peripheral blood samples (20 ml) were collected from each patient into heparinized tubes before the onset of treatment and after a 12-week treatment. Lymphocytes were separated by Ficoll–Hypaque centrifugation. Standard direct-labeling techniques were used as recommended by the manufacturers. Immunophenotyping was done by flow cytometry using conjugated antibodies able to detect specific epitopes. The antibodies for the identification of lymphocyte subsets were OKT4 (CD4) and OKT8 (CD8) from Ortho (Raritan, NJ). Immunofluorescence was examined by a FACScan analyser (Becton Dickinson). The proportion of lymphocytes stained with each monoclonal antibody was converted to the absolute number per microliter by multiplying by the number of lymphocytes per microliter derived from the whole blood count.

2.9. Determination of natural killer (NK) activity

NK cell activity was tested in the total peripheral blood mononuclear cells (PBMC) population against the NK-sensitive K562 tumor cells by means of ⁵¹Cr release assay as previously described [23]. Briefly, effector PBMC were freshly isolated and plated in 100 μl aliquots in 96-well microtiter plates. Tumor target cells (10⁵) were incubated with 100 μCi of sodium ⁵¹Cr chromate (Amersham) for 90 min at 37 °C, washed twice to remove excess isotope and a quantity amounting to 5 × 10⁵ cells/well was added to the effector cells, to assess an effector to target (E/T) ratio of 40 to 1. After 18 h incubation at 37 °C in a CO₂ incubator, 100 μl of supernatant were removed from each well for radioactivity counting using a γ-counter. Spontaneous and maximum release was established by incubating target cells in medium alone or with 5% Triton-X 100, respectively. Spontaneous release did not exceed 15% of the maximum release. In all cases, cultures were set up in triplicate and percentage of (%) specific target cell lysis was calculated as follows:

\[
\% \text{ specific target cell lysis} = \frac{\text{Mean cpm}_{\text{experimental}} - \text{Mean cpm}_{\text{spontaneous}}}{\text{Mean cpm}_{\text{maximum}} - \text{Mean cpm}_{\text{spontaneous}}} \times 100
\]

2.10. Statistical analysis

Data were expressed as the mean ± standard deviation (SD). Correlation analysis was performed to determine the relationship between the immune variables measured (responses changed from the baseline values) in study patients. Comparisons of means of the immune variables at baseline and 12-week treatment were made using Student’s paired samples t-test. A p value of less than 0.05 was adopted as statistically significant.

3. Results

3.1. Mitogenic reactivity to PHA

The G. lucidum extracts at 0.05–1.0 mg/ml produced little or negligible cytotoxicity against peripheral mononuclear cells from cancer patients. There was an 8-fold variability in the mitogenic reactivity to PHA as determined by ³H-thymidine incorporation by peripheral mononuclear cells among 41 patients. As shown in Fig. 1, treatment with G. lucidum for 12 weeks increased ³H-thymidine incorporation by peripheral mononuclear cells in 23 (56.1%) patients, while 18 patients had decreased ³H-thymidine incorporation. Among all 41 assessable cancer patients, the mean and median of ³H-thymidine incorporation at baseline were 0.878 × 10⁵ (SD, 0.316) and 0.883 × 10⁵ cpm and the values were slightly increased to 0.898 × 10⁵ (SD, 0.309) and 0.885 × 10⁵ cpm by week 12 (p > 0.05). Gender and smoking had insignificant effect on the changes of ³H-thymidine incorporation.

3.2. Effect of G. lucidum on plasma cytokine concentrations

There was a 3–9-fold variability in the plasma concentrations of IL-1, IL-2, IL-6, TNF-α and IFN-γ in 41 advanced colorectal cancer patients. The mean values for plasma IL-1, IL-2, IL-6, TNF-α and IFN-γ at baseline

![Fig. 1. Mitotic response of peripheral blood lymphocytes to phytohemagglutinin (PHA) at baseline and after 12-week treatment with G. lucidum polysaccharides in patients with advanced colorectal cancer. The data point is from at least three determinations.](image-url)
were 85.6 ± 21.1, 77.7 ± 20.7, 54.7 ± 18.9, 108.6 ± 42.7, and 65.6 ± 24.9 pg/ml, respectively, and the median values for these cytokines were 84.3, 85.2, 52.1, 109.8, and 62.3 pg/ml, respectively. Treatment with *G. lucidum* for 12 weeks resulted in an increase in the plasma concentrations of IL-2, IL-6, and IFN-γ in 29 (70.7%), 30 (73.2%) and 34 (82.9%) patients, respectively; whereas the levels of both IL-1 and TNF-α were decreased in 30 (73.2%) patients (Fig. 2). After a 12-week treatment with *G. lucidum*, the mean values for plasma IL-1, IL-2, IL-6, TNF-α and IFN-γ were 79.6 ± 21.4, 83.3 ± 20.2, 59.1 ± 17.5, 98.9 ± 33.6, and 70.7 ± 22.9 pg/ml, respectively, and the median values for these cytokines were 76.2, 85.4, 55.6, 100.2, and 68.3 pg/ml, respectively. There was no statistical significance (p > 0.05) when a comparison was conducted between the values at baseline and those after a 12-week treatment with *G. lucidum*. Gender and smoking had insignificant effect on the changes of various cytokines tested.

3.3. Effects on the number and proportion of lymphocyte subsets

There was a remarkable variability (9–12-fold) among 41 cancer patients in the number of each lymphocyte subset at baseline. The mean numbers of CD3, CD4, CD8 and CD56 cells at baseline were 1080.8 ± 482.7, 1172.2 ± 456.5, 340.1 ± 174.5, and 292.6 ± 148.8, respectively, and the medi-

![Fig. 2. Plasma concentrations of IL-1 (A), IL-2 (B), IL-6 (C), TNF-α (D) and IFN-γ (E) at baseline and after 12-week treatment with *G. lucidum* polysaccharides in advanced colorectal cancer patients. Values are the mean ± SD.](image-url)
an values were 1034.3, 1220.2, 312.6, and 253.2, respectively. *G. lucidum* treatment produced an increase of CD3, CD4, CD8 and CD56 cells in 29 (70.7%), 22 (53.7%), 24 (58.5%), and 26 (63.4%) patients, respectively (Fig. 3). The mean numbers of CD3, CD4, CD8 and CD56 cells after a 12-week treatment with *G. lucidum* were 1111.4 ± 430.1, 1191.9 ± 435.9, 346.4 ± 195.1, and 304.5 ± 147.1, respectively, and the median values were 1123.3, 1209.4, 304.3, and 267.5, respectively. However, *G. lucidum* treatment resulted in a decrease of CD4:CD8 ratio in 22 (53.7%) patients (baseline vs 12 weeks: 4.295 ± 3.111 vs 4.194 ± 2.633). There was no statistical significance (*p* > 0.05) when a comparison was conducted between the values at baseline and those after a 12-week treatment. In addition, *G. lucidum* treatment had no or little effect on total peripheral blood lymphocyte counts and the CD4:CD8 T cell ratios.

### 3.4. Effect of *G. lucidum* on NK activity

Baseline NK activity against K562 was evaluated in all 41 colorectal cancer patients and compared to the cytotoxic activity after treatments. There was a marked inter-individual variability among patients in NK cytotoxic activity against K562 target cells as demonstrated by a range of baseline killing varying from 10.3% to 65.3% at a 40:1 effector:target ratio, with a mean and median of 29.5 ± 10.4% and 26.5%, respectively. *G. lucidum* treatment resulted in an increase in the NK activity in 28 (68.3%) patients and the mean and median values were 31.3 ± 9.7% and 30.2%, respectively (Fig. 4). However, a comparison between the values at baseline and those after a 12-week treatment with *G. lucidum* did not show any statistical significance (*p* > 0.05).

### 3.5. Correlations among the immune variables tested

A correlation analysis was conducted to identify the relationships between the changes of these immune functions following *G. lucidum* treatment for 12 weeks. The results are summarised in Table 2. The changes of IL-1 were corre-
lated with those for IL-6, IFN-γ, CD3, CD4, CD8 and NK activity \((p<0.05)\) and IL-2 changes were correlated with those for IL-6, CD8 and NK activity (Fig. 5). The changes of TNF-α were correlated with those for CD3, CD8, CD56, and IFN-γ correlated with CD4. The CD3 changes were correlated with those for CD8 and CD56, whereas the CD4 changes were correlated with thymidine incorporation and NK activity (Fig. 5). Moreover, the changes of CD4:CD8

Table 2
Correlation matrix for the immune variables measured (responses changed from the baseline values) in study patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>IL-1</th>
<th>IL-2</th>
<th>IL-6</th>
<th>TNF-α</th>
<th>IFN-γ</th>
<th>CD3</th>
<th>CD4</th>
<th>CD8</th>
<th>CD56</th>
<th>TI</th>
<th>NK</th>
<th>CD4:CD8</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td></td>
<td>0.2240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>0.2240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>0.3550*</td>
<td>0.4648*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.2478</td>
<td>0.1484</td>
<td>0.2591</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>0.4055*</td>
<td>0.2774</td>
<td>0.2048</td>
<td>0.1765</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD3</td>
<td>0.3320*</td>
<td>0.2527</td>
<td>0.2377</td>
<td>0.3645*</td>
<td>0.2198</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4</td>
<td>0.4376*</td>
<td>0.2099</td>
<td>0.2445</td>
<td>0.1919</td>
<td>0.3348*</td>
<td>0.1688</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD8</td>
<td>0.2887*</td>
<td>0.3407*</td>
<td>0.1693</td>
<td>0.3682*</td>
<td>0.0895</td>
<td>0.4286*</td>
<td>0.0072</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD56</td>
<td>0.0318</td>
<td>0.055</td>
<td>0.0055</td>
<td>0.0750</td>
<td>0.3890*</td>
<td>0.2101</td>
<td>0.392*</td>
<td>0.0715</td>
<td>0.2571</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TI</td>
<td>-0.0549</td>
<td>0.1124</td>
<td>0.2476</td>
<td>0.2459</td>
<td>0.2256</td>
<td>0.0452</td>
<td>0.3786*</td>
<td>0.0844</td>
<td>0.3652*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NK</td>
<td>0.4570</td>
<td>0.3352*</td>
<td>0.2288</td>
<td>0.0621</td>
<td>0.2428</td>
<td>0.4683*</td>
<td>0.1272</td>
<td>0.0052</td>
<td>-0.0007</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4:CD8</td>
<td>0.2271</td>
<td>0.0936</td>
<td>0.2483</td>
<td>0.0756</td>
<td>0.3062*</td>
<td>-0.0355</td>
<td>0.7761*</td>
<td>-0.5028*</td>
<td>0.0101</td>
<td>0.3784*</td>
<td>0.3455*</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.05. TI=thymidine incorporation.

Fig. 5. Correlations between the immune variables tested in patients with advanced colorectal cancer. Patients were treated with \(G. \ lucidum\) polysaccharides for 12 weeks. Values are the mean ± SD.
ratio were correlated with those for IFN-γ, CD4, thymidine incorporation and NK activity.

4. Discussion

The present study indicated that treatment of advance colorectal patients with *G. lucidum* extracts at 5.4 g/day for 12 weeks tended to enhance the lymphocyte mitogenic reactivity to PHA, counts of CD3, CD4, CD8 and CD56, and NK activity, but statistical significance was not observed. The treatment with *G. lucidum* also tended to increase IL-2, IL-6 and IFN-γ but decrease IL-1 and TNF-α in the study patients. Minimal effects, limited sample size and marked individual variability in the variables measured might all contribute to the lack of statistical significance. Our study demonstrated that the changes of the immune variables monitored were correlated. For example, the changes of IL-1 were correlated with those for IL-6, IFN-γ, CD3, CD4, CD8 and NK activity (p<0.05) and IL-2 changes were correlated with those for IL-6, CD8 and NK activity. This indicated that the immune variables monitored through this study were all important effector components interplayed in the network of native defense.

The fate of the host–tumor interactions is considered to depend on the balance between the intrinsic aggressiveness (i.e. inherent metastatic potential) of the tumor and the strength of the host immune response. Cytotoxic T lymphocyte and NK activity play an important role in immunological surveillance in neoplasia and metastasis [24,25]. Cytotoxic T lymphocytes have been shown to recognize specifically antigenic peptides in the context of major histocompatibility complex Class I molecules, while the ligands for triggering NK receptors involved in natural cytotoxicity are still largely unclear [26]. Human NK cells, comprising approximately up to 15% of all circulating lymphocytes, can cause early production of cytokines and chemokines (e.g. ILs) and lyse tumor cells without prior sensitization [24]. In the late stage of cancer patients, NK activity is significantly decreased and associated with an impairment of cytokine production [27,28]. Low NK activity has been associated with poor prognosis in advanced cancer patients [29]. Therefore, a number of immunotherapeutic approaches aimed to enhance NK activity and production of cytokines such as IL-2 have been investigated in advanced-stage cancer patients and beneficial effects have been observed in some patients [30]. In addition to active and passive transfer of functional NK cells and cytokines, natural medicines may be an important complement of these approaches. This study indicated that Ganopoly increased NK cell number and activity in 63.4–68.3% patients with advanced colorectal cancer. Various natural medicines such as Transfer Factor Plus and *Agaricus blazei* Murill teas significantly increased NK activity in humans [31].

Macrophages are involved in all stages of the immune response. They act as a rapid protective mechanism by engulfing exogenous particles prior to T cell activation; take part in the initiation of T cell activation by processing and presenting antigen; and finally act as inflammatory, tumoricidal and microbicidal cells in the effector phase of the cell-mediated responses following T-cell mediated activation. Macrophages have been found to play a key role in the activity of anti-tumor of *G. lucidum* PS. The β-D-glucans present in *G. lucidum* PS can bind membrane complement receptor type three (CR3, αMβ2 integrin, or CD11b/CD18) on macrophages [32,33]. CR3 has been mapped to a region of CD11b-located C-terminal to the I-domain and its distinct metal ion-dependent adhesion site for the many protein ligands of CR3 such as iC3b, ICAM-1 and fibrinogen [34,35]. The binding of β-D-glucans to CR3 will activate the macrophages, resulting in induced release of cytokines (e.g. IL-1β, IL-6, IFN-γ and TNF-α), nitric oxide and other mediators [9,34]. All these cytokines can induce apoptosis of tumor cells [9]. The β-D-glucans consist of a linear backbone of β-(1→3)-linked D-glucopyranosyl groups with varying degrees of branching from the C6 position.

The immune function can be reduced or damaged in many cancer patients when treated with chemotherapy and radiotherapy, negating the therapeutic benefits obtained by the increased tumor killing of the treatment [23]. These negative effects may be particularly important in the treatment of immunogenic tumors where immunological function of the host is a determining factor for the clinical outcome of treatment. Thus, combination of chemotherapy or radiotherapy with immunomodulating agents may provide a strategy for overcoming the immunosuppressive effects of chemotherapy/radiotherapy. Recently, many biological response modifiers have been combined with cytotoxic chemotherapeutic agents/radiation, in attempt to enhance anticancer activity and reduce toxicity. It would be expected that *G. lucidum* would negate the immunosuppressive effects of traditional chemotherapy/radiotherapy in cancer patients.

*G. lucidum* treatment for 12 weeks resulted in a decrease of TNF-α and IL-1 in 73.2% of study patients. The inhibitory effects of *G. lucidum* on TNF-α and IL-1 production may result in beneficial effects (e.g. improved life quality) in advanced-stage cancer patients.
Increased cytokines (e.g. TNF-α and IL-1) have been thought to contribute to cancer cachexia that is manifested by bodyweight loss, chronic nausea, fatigue, insomnia and profuse sweating [36]. Drugs that down-regulate TNF-α and IL-1 can result in improvements of cancer cachexia [37]. Thus, *G. lucidum* may represent a useful approach to improve cancer cachexia.

It is important but difficult to establish the exposure time–effect relationships of *G. lucidum* polysaccharides. The treatment regimen for 3 months (12 weeks) was based on folk medicine use of *G. lucidum*. Longer period of administration with *G. lucidum* polysaccharides may result in significant outcome, but potential side effects are also a concern when long-term regimen is used. Cases have been reported where long-term treatment of oral *G. lucidum* polysaccharides induced allergy (e.g. rash and asthma) and gastrointestinal side effects (e.g. nausea, vomiting, and diarrhea) [38].

Overall, the findings from the present study suggest that *G. lucidum* may act as a host defense potentiator. It may represent a practical and promising adjunct approach for cancer treatment in combination with chemotherapy/radiotherapy. However, in future study, with regard to clinical evidence, the issues of duration of intervention and primary outcome measures with a more rigorous design should be considered as changes to specific cytokines may not be as important as the improvement of quality of life given that the exact mechanisms of action of *G. lucidum* are yet to be elucidated.

Acknowledgments

The authors gratefully appreciate the support by the National University of Singapore Academic Research Funds and Encore International Corporation, Auckland, New Zealand.

References