Abstract. Distant metastasis is one of the major problems in treatment for advanced colorectal cancer. Polysaccharide-K (PSK), or Krestin, a mushroom ingredient, has been used as a chemoimmunotherapeutic agent for the treatment of cancers in Asia for over 30 years. Some studies have reported that PSK prevent distant metastases and improve survival rates by 10-20% in colorectal cancer. However, the mechanism of the interrelated immunomodulatory and direct anti-cancer cell activities of PSK has yet to be elucidated. To investigate the direct effect, we used cDNA microarrays to analyse expression profiles in a human colorectal adenocarcinoma cell line, HCT116, containing the wild-type p53 gene. Expression of 453 genes was significantly altered (142 up-regulated and 311 down-regulated) after 96 h exposure to 500 \( \mu \)g/ml PSK. Under more stringent conditions, 9 genes were up-regulated and 36 down-regulated. We then examined the expression of candidate genes in two cell lines, HCT116, and SW480, a cell line with a mutant p53 gene. Our results suggest that PSK may augment anti-tumour action via genes including multidrug resistance protein 3 (MRP3), lymphotactin (Lptn), transgelin (TAGLN), and Pirin, without disturbing cell-cycle progression, and may deserve a large clinical trial in cancer therapy.

Introduction

Administration of fluorouracil, commonly modulated by calcium folate, is now the standard therapy for advanced colorectal cancer; however, treatment yields objective responses in only 20-30% of patients (1,2). Once metastatic disease is diagnosed, the 5-year survival rate is <5%. In the majority of cases, chemotherapy is the recommended treatment for patients with advanced metastatic disease, but quality of life in patients receiving this treatment is generally poor (3). A trend towards the integration of immunopotentiating agents with the extant treatment regimens of surgery, chemotherapy, and radiation therapy has gained popularity as an adjuvant therapy for cancer during the last three decades.

Intriguingly, total visits to alternative medicine practitioners in the United States increased by 47.3% between 1990 and 1997, reaching 629 million in 1997, and thereby exceeding the total number of visits to all US primary care physicians. At the same time, the estimated expenditure for alternative medicine associated professional services increased by 45.2% between 1990 and 1997 and were conservatively estimated at $21.2 billion in 1997, with at least $12.2 billion paid out-of-pocket. This exceeded the 1997 out-of-pocket expenditures for all US hospitalisations (4,5). According to a Japanese newspaper, about one-third of cancer patients in Japan also take supplements, including traditional ingredients for general well-being (e.g., mushrooms), as biological response modifiers (BRMs) without telling their physicians. The benefits for cancer of non-specific immunopotiation with BRMs, i.e., OK-432, BCG, polysaccharides, and endogenous cytokines, are thought to be due to 1) immunomodulatory effects, 2) direct anti-neoplastic effects, 3) chemotherapeutic protection of normal tissue, and 4) restorative effects in patients who have been immunosuppressed by both recent surgery and subsequent chemotherapy (6). However, despite their popularity, as yet there has been no scientific evaluation of the effectiveness of these BRMs in cancer.

Polysaccharide-K (PSK), or Krestin, a protein-bound polysaccharide, is a BRM prepared from the mushroom Coriolus versicolor, and has been used in traditional Chinese medicine for centuries (7). PSK (Sankyo Co., Tokyo, Japan) is widely used in adjuvant therapy after surgery or radiotherapy in Japan and other Asian countries, and the Japanese National Health Insurance scheme covers the use of PSK for gastric, colorectal, and lung cancers. Surprisingly, randomised controlled clinical studies have revealed that PSK in adjuvant therapy for gastric, colorectal, esophageal, and lung cancers.
significantly extends the 5-year survival rates of patients by 10-20% (8-11). Compared with colorectal cancer patients who did not receive PSK, patients receiving PSK showed a lower local recurrence rate (9.5% in the PSK group versus 11.9% in non-PSK group), systemic recurrence rate (16.3% in PSK group versus 19.8% in non-PSK group), lymph node recurrence rate (1.8% in PSK group versus 5.3% in non-PSK group), and peritoneal dissemination rate (1.8% in PSK group versus 3.5% in non-PSK group), and a higher 3-year survival rate (77.2% in PSK group versus 67.7% in non-PSK group) (9). PSK produces very few serious adverse side effects, and its characteristics allow long-term oral administration. The anti-tumour actions of PSK are considered to be not only host-mediated but also to involve a direct regulatory action on the growth factor production and enzyme activities of tumours (17,18). To unravel the mechanism that induces anti-neoplastic immunity, we investigated alterations in gene expression that are potentially required for direct action of PSK, using a cDNA microarray containing 8581 human cDNA fragments in a human colorectal adenocarcinoma cell line, HCT116, with a wild-type p53 gene. Mutations of the p53 gene have been found in approximately 50% of colorectal carcinomas and are associated with lymphatic dissemination and a poorer prognosis (19,20). We therefore examined alterations in expression of candidate genes in HCT116 cells, and in SW480 cells which have a mutant p53 gene, before and after exposure to PSK.

Materials and methods

Cell culture and PSK treatment. A colorectal adenocarcinoma cell line, HCT116, with a wild-type p53 gene was purchased from the American Type Culture Collection (Manassas, VA, USA), and a cell line with a mutant p53 gene, SW480, was obtained from the Human Science Research Resource Bank (Tokyo, Japan). The cells were grown in RPMI-1640 medium (Gibco, Grand Island, NY, USA) supplemented with fetal bovine serum (10% (v/v), HyClone, Logan, UT, USA), glucose (2 mM), penicillin (100,000 units/l), streptomycin (100 µg/l), and gentamycin (40 µg/ml). Cell cultures were maintained at 37˚C, in a humidified atmosphere of 5% CO2/95% air. For the cell growth study, 106 cells were plated per 60 mm dish and treated with PSK (Kureha Chemical Co., Tokyo, Japan) at the indicated concentrations. Cells were counted using a haemocytometer on the days indicated. Cells were then prepared for flow cytometry, isolation of total cell RNA and cDNA analysis using Atlas™ microarrays (Clontech laboratories, Inc. Palo Alto, CA, USA).

Flow cytometry. Floating and trypsinized adherent cells were collected, suspended in PBS (-), fixed with 70% (v/v) ethanol, and stained with propidium iodide (50 µg/ml). DNA content was analysed using a FACScan (Becton-Dickinson, Lincoln Park, NJ, USA) with CellQuest and Mod Fit LT 1.0 software (Verity Software House, Inc., Topsham, ME, USA). Cell debris and fixation artifacts were gated out.

Isolation of RNA. After treatment, cell lines grown to log phase as monolayers were washed twice with PBS and total RNA was extracted using Isogen (Nippon Gene, Tokyo, Japan), an acid guanidine thiocyanate-phenol-chloroform method. Isolated RNA was electrophoresed through 1.0% agarose-formaldehyde gels to verify the quality of the RNA, and RNA concentrations were determined from absorption measurements at 260 and 280 nm.

Identification of gene expression profiles by cDNA microarray. cDNA synthesis, microarray hybridisation, scanning, grid-assisted spot identification, and analysis were performed according to the manufacturer’s instructions (Clontech laboratories, Inc.). Briefly, 100 µg of total cellular RNA was primed with oligo(dT) and reverse-transcribed in the presence of Cy3-labeled or Cy5-labeled dATP, using 1 µl of MMLV reverse transcriptase. The resulting Cy3- and Cy5-labeled cDNAs were treated with RNase I for 10 min at 37˚C, combined, purified using a NucleoSpin®, Extraction Spin Column, and assessed for radioactivity by scintillation counting. Sample and control-labelled probes were mixed together and hybridised to cDNA microarray slides containing 8581 human genes in the Atlas Plastic Human 8K (#7905-1) and Human Cytokine/Receptor (#7744-1) Arrays. Names of these genes are available at http://www.clontech.com/atlas/genelists/index.shtml. The hybridisation solution, ExpressHyb, was placed on a pre-treated microarray slide, and then incubated in a hybridisation chamber overnight at 50˚C. After hybridisation, the slide was washed at room temperature, first with 0.2X SSC, 0.1% SDS for 20 min with gentle shaking, then twice with 0.2X SSC (20 min each time). Hybridised slides were scanned, the scanner output images were localised by overlaying a grid on the fluorescent images, and these were then analysed using the AtlasImage™ software. Each slide contained 9 housekeeping genes to normalise the signal intensities of the fluorescent dyes. The intensities of Cy5 and Cy3 were adjusted so that the mean Cy5 and Cy3 intensities of probe cDNA binding to the housekeeping genes were equivalent. Both final reported intensities (green and red) were filtered, and the spots with intensity <1.5 were eliminated. The ratios of red to green and green to red intensities for all targets were determined. The cDNA microarray results comparing cells treated with or without PSK are based on two completely independent experiments involving separate cell treatments, separate RNA isolations, and separate microarray assays.

RT-PCR. First-strand cDNA synthesis was performed with 20 µg of total RNA using a first-strand DNA synthesis kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). Standard RT-PCR procedures were carried out in 20 µl volumes of PCR buffer and amplified for 5 min at 95˚C for denaturing, followed by 25 (for GAPDH) or 35 (for others) cycles of 95˚C for 30 sec, 62˚C (for GAPDH, Lptn, and integrin), 64˚C (for TAGLN, and T54), 66˚C (for Pirin), or 68˚C (for MRP3) for 30 sec, and 72˚C for 30 sec. Two sets of PCR primers were used, including the sense (5'-GAAGATG
TCCTAAGCCACGATA-3’) and antisense (5’-GCAACTTTCTCATATGTTACAGA-3’) primers for T54, the sense (5’-GACATGGCAGCAGTGCAGAG-3’) and antisense (5’-CAGGGCTGAAGGTACCACAC-3’) primers for transgelin (TAGLN), the sense (5’-GCCAAACATTCCCAACCTACT-3’) and antisense (5’-CTAGGACACATCAAGACCTGCT-3’) primers for Pirin, the sense (5’-GGGACCCTGCGCTGAACCTG-3’) and antisense (5’-AGGCAAGTCCAGCATCTCTGG-3’) primers for multidrug resistance protein 3 (MRP3, also known as ATP-binding cassette, sub-family C, member 3, ABCC3), the sense (5’-TTACACATCAGTCACAAGTTACAG-3’) and antisense (5’-GCATAACATGCATGATAACTAGCG-3’) primers for the lymphotactin (Lptn) receptor gene XCR-1, the sense (5’-CAGCAACGGCAGATCTGCA-3’) and antisense (5’-GAACCAACAGTCGTCAACATC-3’) primers for integrin ß1, and the sense (5’-CGGAGTCAACGGATTTGGTCGTAT-3’) and antisense (5’-AGCCTTCTCCATGGTGGTGAAGAC-3’) primers for the control gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). All PCR reaction products were analysed on ethidium bromide-stained 3% agarose gels and photographed under ultraviolet light.

Results

Suppression of cell growth by PSK. We examined the effects of PSK at various concentrations (0-1,000 µg/ml) on the growth of two colorectal carcinoma cell lines containing wild-type p53 (HCT116) and mutant p53 (SW480). Exposure to 500 and 1000 µg/ml of PSK suppressed the growth of HCT116 cells by 58.9 and 51.6% at 96 h, respectively (Fig. 1), and of SW480 cells by 69.5 and 60.8% at 96 h, respectively (data not shown).

Flow cytometry. Neither HCT116 nor SW480 cells showed any marked difference in cell-cycle distribution pattern before or after treatment with 500 µg/ml PSK for 96 h, while the proportion of apoptotic cells, which appeared in a region to the left of the G1 peak, increased from 3.6 to 12.9% in HCT116 cells after treatment (Fig. 2), and from 2.8 to 9.8% in SW480 cells (data not shown).

Analysis of expression profiles. Gene expression was analysed in HCT116 cells following 96 h of treatment with 500 µg/ml PSK (Atlas Plastic Human 8K Arrays #7905-1). Under basic selection conditions, a total of 453 genes were selected from 8,581 human genes as elements associated with exposure to PSK in HCT116 cells. These genes were identified on the basis of altered expression with two-fold or higher ratios, and included 142 up-regulated and 311 down-regulated genes. Further analyses of gene expression patterns allowed us to identify 45 genes showing a four-fold or more change in expression, including 9 up-regulated and 36 down-regulated genes (Table I). Genes showing up-regulated expression included MRP3, lymphotactin, and integrin ß1 in order of increasing ratio. Genes showing down-regulation included T54 protein, transgelin, and Pirin in order of decreasing ratio. Following exposure to 500 µg/ml PSK for 24 or 96 h, no significant change could be identified in any of the cytokines we examined (Atlas Human Cytokine/Receptor Arrays #7744-1).

Semi-quantitative analysis of MRP3, Lptn, integrin, T54, TAGLN, and Pirin mRNA expression in HCT116 and SW480 cell lines. The 3 genes exhibiting the greatest positive regulation, and the 3 showing the greatest negative regulation, were selected for further analysis by RT-PCR (Fig. 3). Expression was assessed at 0, 6, 12, 24 and 96 h after treatment with 500 µg/ml PSK. MRP3 mRNA expression gradually decreased over 24 h by 0.6- and 0.75-fold, and then increased at 96 h by 3.4- and 2.5-fold in HCT116 and SW480 cells, respectively, as compared to controls without exposure to PSK. Lptn mRNA expression increased after 12 h both in HCT116 and SW480 cells (3.2- and 3.4-fold, respectively, of the control level at 96 h). No significant change was observed in any of the cytokines we examined (Atlas Human Cytokine/Receptor Arrays #7744-1).
Table I. Representative differential gene expression defined by a 4-fold or greater change in signal intensity, induced by 500 µg/ml PSK treatment for 96 h in the HCT 116 cell line.

<table>
<thead>
<tr>
<th>Genebank #</th>
<th>Definition</th>
<th>Spot intensity</th>
<th>Genebank #</th>
<th>Definition</th>
<th>Spot intensity</th>
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<tr>
<td>NM_003786</td>
<td>ATP-binding cassette, sub-family C (CFTR/MRP), member 3</td>
<td>Control 4</td>
<td>PSK treated 33</td>
<td>Cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMP-N-acetylneuraminic monoxygenase)</td>
<td>Control 54</td>
</tr>
<tr>
<td>NM_002995</td>
<td>Small inducible cytokine-subfamily C, member 1 (lymphotactin, Lptn)</td>
<td>Control 12</td>
<td>PSK treated 93</td>
<td>Vascular endothelial growth factor</td>
<td>Control 19</td>
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<tr>
<td>X07979</td>
<td>Integrin, β1 (fibronectin receptor, β polypeptide, antigen CD29 includes MDF2, MSK12)</td>
<td>Control 10</td>
<td>PSK treated 54</td>
<td>Glutamate receptor, ionotropic, kainite 1</td>
<td>Control 19</td>
</tr>
<tr>
<td>NM_003168</td>
<td>Suppressor of Ty (S, cerevisiae) 4 homolog 1</td>
<td>Control 13</td>
<td>PSK treated 72</td>
<td>81,3-N acetylgulosaminyltransferase</td>
<td>Control 7</td>
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<td>NM_012206</td>
<td>hepatitis A virus cellular receptor 1</td>
<td>Control 5</td>
<td>PSK treated 26</td>
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<td></td>
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<tr>
<td>NM_015698</td>
<td>T54 protein</td>
<td>Control 30</td>
<td>PSK treated 0</td>
<td></td>
<td>Control 25</td>
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<tr>
<td>NM_003186</td>
<td>transgelin (TAGLN)</td>
<td>Control 37</td>
<td>PSK treated 1</td>
<td></td>
<td>Control 15</td>
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<tr>
<td>NM_003662</td>
<td>Pir</td>
<td>Control 17</td>
<td>PSK treated 1</td>
<td></td>
<td>Control 15</td>
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<tr>
<td>NM_003905</td>
<td>Amyloid β precursor protein-binding protein 1, 59kD</td>
<td>Control 26</td>
<td>PSK treated 2</td>
<td>Protein ‘A’</td>
<td>Control 107</td>
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<tr>
<td>D 11086</td>
<td>Interleukin 2 receptor, gamma (severe combined immunodeficiency)</td>
<td>Control 13</td>
<td>PSK treated 1</td>
<td>N-acylaminoacyl-peptide hydrolase</td>
<td>Control 41</td>
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<tr>
<td>NM_014481</td>
<td>Apurinic/apyrimidinic endonuclease (APEXnuclease)-like 2 protein</td>
<td>Control 36</td>
<td>PSK treated 3</td>
<td>Baculoviral 1AP repeat-containing 5 (surviving)</td>
<td>Control 108</td>
</tr>
<tr>
<td>NM_014463</td>
<td>Lsm 3 protein</td>
<td>Control 15</td>
<td>PSK treated 15</td>
<td>CGI-27 protein</td>
<td>Control 18</td>
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<tr>
<td>NM_003518</td>
<td>H2B histone family, member A</td>
<td>Control 18</td>
<td>PSK treated 2</td>
<td>TNF receptor-associated factor 2</td>
<td>Control 18</td>
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<tr>
<td>NM_014564</td>
<td>LJM homeobox protein 3</td>
<td>Control 17</td>
<td>PSK treated 2</td>
<td>CDC 20 (cell division cycle 20, S. cerevisiae, homolog)</td>
<td>Control 218</td>
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<tr>
<td>NM_004655</td>
<td>Axin 2 (conductin, axil)</td>
<td>Control 15</td>
<td>PSK treated 2</td>
<td>Thymidine kinase 1, soluble</td>
<td>Control 81</td>
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<td>NM_004219</td>
<td>Pituitary tumor-transfoming 1</td>
<td>Control 43</td>
<td>PSK treated 6</td>
<td>Insulin induced gene 1</td>
<td>Control 30</td>
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<tr>
<td>NM_004153</td>
<td>Origin recognition complex, subunit 1 (yeast homolog)-like</td>
<td>Control 36</td>
<td>PSK treated 5</td>
<td>Zinc finger protein 177</td>
<td>Control 30</td>
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<tr>
<td>NM_016359</td>
<td>Clone HQ0310 PRO0310p1</td>
<td>Control 14</td>
<td>PSK treated 2</td>
<td>RecQ protein-like 4</td>
<td>Control 26</td>
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<tr>
<td>NM_001034</td>
<td>Ribonucleotide reductase M2 polypeptide</td>
<td>Control 303</td>
<td>PSK treated 49</td>
<td>Mitotic spindle coiled-coil related protein</td>
<td>Control 17</td>
</tr>
<tr>
<td>NM_015932</td>
<td>Hypothetical protein</td>
<td>Control 24</td>
<td>PSK treated 4</td>
<td>KIAA0670 protein/acinus</td>
<td>Control 25</td>
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<tr>
<td>NM_003504</td>
<td>CDC45 (cell division cycle 45, S. cerevisiae, homolog-like)</td>
<td>Control 35</td>
<td>PSK treated 6</td>
<td>Polymerase (RNA) III (DNA directed) (23 kD)</td>
<td>Control 41</td>
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<tr>
<td>NM_007317</td>
<td>Kinesin-like-4</td>
<td>Control 45</td>
<td>PSK treated 8</td>
<td>DEAD/H (Asp-Glu Ala-AspHis) box polypeptide 8 (RNA helicase)</td>
<td>Control 28</td>
</tr>
<tr>
<td>NM_004308</td>
<td>Rho GTPase activating protein 1</td>
<td>Control 43</td>
<td>PSK treated 8</td>
<td>Budding uninhibited by benzimidazoles 1 (yeast homolog), β</td>
<td>Control 20</td>
</tr>
</tbody>
</table>

The upper section of the table shows genes which were increased with PSK treatment, while the lower part shows those that were decreased. Genes are ranked in increasing or decreasing order of fold-change. The 3 genes exhibiting the greatest regulation, and the 3 showing the greatest negative regulation, were selected for further study; these are indicated in bold.
Our results have shown that PSK can have an additional combination with the standard PMC regimen (unpublished). Decreasing after the introduction of PSK therapy in a group versus 18.4 months in non-PMC group, p=0.00002 (median liver metastasis free time; 34.2 months in PMC regimen seems to decrease liver metastasis significantly could not be reduced by PMC only, although our PMC cancer (23). Extrahepatic recurrence, mostly in the lung, recurrence by 49% in patients with stage II or III colorectal UFT (tegafur/uracil), a 5-FU derivative, reduces the risk of cancer cell characteristics or tolerance of individual patients, especially in those with advanced colorectal cancers whose general condition is poor. The genes described here could conceivably be strong candidates for molecular targets of PSK in chemoimmunotherapy.

Our study showed that the cytotoxic effects of PSK were attributable to apoptosis via a p53-independent pathway. Furthermore, analyses of gene expression patterns of colorectal adenocarcinoma cells with or without PSK treatment allowed us to identify 45 genes showing changes in expression of four-fold or more. We also confirmed altered expression of MRP3, Lptn, TAGLN, and Pirin mRNA in both cell lines with wild-type or mutant p53 genes. Unexpectedly, the results obtained from the RT-PCR experiments for 96 h did not agree with those from the cDNA microarray at the one time-point of 96 h. In particular, integrin ß1 and T54 failed to confirm any effect of PSK on expression. This fact suggests that cDNA microarray analysis at one time point may not be informative enough for identifying the crucial target genes and should be utilised only for preliminary screening of potential candidate genes. Further investigation into the effect of PSK treatment on expression of integrin ß1 and T54 is also required.

MRP3 has been shown to mediate ATP-dependent transport of organic anions including 17ß-glucuronosyl oestradiol, glucuronosyl bilirubin, and monovalent and sulfated bile salts (26,27). The MRP family consists of several members and, for some of these transporter proteins, distinct roles have been well established in multidrug resistance and normal tissue functions (MRP1 and MRP2) or are still under investigation (MRP3). MRP3 has been reported to be induced by sulindac; a nonsteroidal anti-inflammatory drug (NSAID), via redox regulation, independent of cyclooxygenase-2 (28). Taking into consideration the involvement of NSAIDs in anti-neoplastic and anti-inflammatory effects in the colon and rectum, the induction of MRP3 by PSK might effect cancer cells per se directly in the same manner as NSAIDs. Alternatively, exploiting the decrease of MRP3 by 24 h exposure to PSK might be a contributing factor in preventing the acquisition of anti-cancer drug resistance during chemotherapy.

Lptn is a C chemokine defined in 1994 that specifically regulates the migration of T-cells and NK cells (29,30). Cotransfection of Lptn and IL-2 genes into tumour tissue could potentiate anti-tumour immunity (31). Adenoviral delivery of Lptn and either IL-2 or IL-12 synergised to facilitate tumour regression in murine breast adenocarcinoma models (32). Lptn gene-modified dendritic cell (DC) based

### Discussion

We have had clinical success against colorectal cancer with ‘Pharmacokinetic Modulating Chemotherapy (PMC)’ which was designed as a hybrid of lower continuous and higher shorter plasma 5-FU concentration (cumulative 5-year survival rate of Dukes’ C colorectal cancer patients; 95% in PMC group versus 67% in non-PMC group, p=0.003) (21). PMC provides remarkable advantages including less toxicity and lower cost through outpatient treatment. Recently, we have reported that this efficacy is gained from exploiting dual cell cycle checkpoint regulation at the G1/S and G2/M transitions (22). The next step will be to increase the efficacy of the extant regimen by potentiating anti-tumour immunity biological response modification, thus exploiting the molecular targets, because there are always a few patients who show poor prognosis regardless of vigorous anti-cancer therapy. Ohwada et al have also shown that oral PSK with UFT (tegafur/uracil), a 5-FU derivative, reduces the risk of recurrence by 49% in patients with stage II or III colorectal cancer (23). Extrahepatic recurrence, mostly in the lung, could not be reduced by PMC only, although our PMC regimen seems to decrease liver metastasis significantly (median liver metastasis free time; 34.2 months in PMC group versus 18.4 months in non-PMC group, p=0.00002) (24). In contrast, extrahepatic recurrences are significantly decreasing after the introduction of PSK therapy in combination with the standard PMC regimen (unpublished data). Our results have shown that PSK can have an additional effect on cancer cells per se without disturbing the cell cycle distribution. PSK might additionally alter the local characteristics of tissue specific factors as well as the direct effect on cancer cells. Recently, Müller et al have reported that some chemokines, released in large quantities from certain organs, can attract circulating cancer cells to take up residence there, leading to the bias of secondary tumours’ location (25). Efficient induction of specific and non-specific anti-tumour immunity, both locally and systemically, might be contributory for inhibiting the survival of tumour cells with invasive capacity. A chemotherapeutic regimen utilising alternative medicine such as PSK can be applied as an integrated therapy without consideration for the different cancer cell characteristics or tolerance of individual patients, especially in those with advanced colorectal cancers whose general condition is poor. The genes described here could conceivably be strong candidates for molecular targets of PSK in chemoimmunotherapy.

![Figure 3. RT-PCR analysis of MRP3, Lptn, integrin ß1, T54, TAGLN, and Pirin mRNA expression by PSK in HCT116 and SW480 cell lines. The amplification products had the expected sizes of 452 bp for MRP3, 321 bp for Lptn, 301 bp for integrin, 343 bp for T54, 346 bp for TAGLN, and 371 bp for Pirin. An amplified fragment of GAPDH demonstrated integrity of the isolated RNA and was used for standardization of candidate genes expressions as an internal control.](image)
tumour vaccines potentiated the anti-tumour effects in mice with lung carcinoma and melanoma (33). Adenovirus-mediated Lptn gene transfer followed by administration of 5-fluorouracil, a premetabolite of 5-FU, elicited potent anti-tumour effects in a pre-established murine colon carcinoma (34). In addition, local expression of Lptn within tumours increased infiltration of CD4+ and CD8+ lymphocytes, as well as neutrophils, leading to the eradication of well-established tumour masses (35,36). Furthermore, allogeneic tumour cell vaccines combining transgenic Lptn with IL-2 showed little toxicity and augmented an anti-tumour immune response, including a few cases of complete remission in humans (37). Taken together, Lptn appears to play a crucial role in recruiting T-cells, resulting in increased exposure to tumour antigens displayed on the dedicated antigen-presenting cells, although T-cell migration alone is not associated with an increase in immunological activity, as measured by an anti-tumour effect.

Transgelin was identified previously as a smooth muscle protein overexpressed in senescent human fibroblasts (38). A recent report suggests that loss of transgelin gene expression through a Ras-independent mechanism may be an early event in tumour progression and a diagnostic marker for breast and colon cancer development (39). The possibility that induction of transgelin by PSK might reduce the invasive properties of colorectal cancer cells cannot be excluded, although no change was confirmed in re-expressed transgelin in Ras-transformed cells.

Pirin interacts with the nuclear factor I/C/CAAT box transcription factor (NFIC/CTF1), which is known to stimulate adenovirus DNA replication and RNA polymerase II-driven transcription (40). Human Pirin stabilises the formation of quaternary complexes between the human oncogene Bel-3, the anti-apoptotic transcription factor NF-kB and its DNA target sequences in vitro (41). Interestingly, Le-pirin, a tomato homologue of human Pirin, dramatically increases during camptothecin-induced cell death (42).

In conclusion, this study provides initial evidence of the candidate gene profiles of anti-neoplastic intervention by PSK, a mushroom ingredient that has long been used in traditional Chinese medicine. The strategy exploiting immune augmentation based on the molecular sketch outlined here would be a contributing factor for those patients.

Acknowledgements

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