Gene expression in response to anti-tumour intervention by polysaccharide-K (PSK) in colorectal carcinoma cells

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Received July 2, 2004; Accepted September 9, 2004

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 μg/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 immunopotentiation 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

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Key words: multidrug resistance protein 3, lymphotactin, transgelin, Pirin, pharmacokinetic modulating chemotherapy (PMC), complementary and alternative medicine (CAM)

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-neoplastic effects of PSK have also been reported in animal models, and shown to involve radical trapping and modulation of cytokine production and effector cell functions (12-13). *In vitro* studies confirmed that PSK induces gene expression of several cytokines including *TNF-α*, *IL-1*, *IL-2*, *IL-4*, *IL-6*, *IL-7*, and *IL-8*, amongst others (14-16).

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), glutamine (2 mM), penicillin (100,000 units/l), streptomycin (100 μg/l), and gentamycin (40 mg/l). Cell cultures were maintained at 37˚C, in a humidified atmosphere of 5% CO_2/95% air. For the cell growth study, 10^6 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 absorbance 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

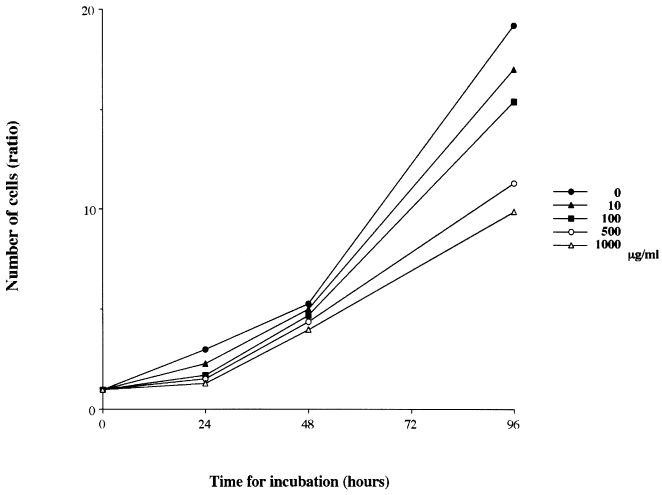

Figure 1. The growth curve of HCT116 following continuous exposure to 1-1000 μg/ml PSK. ●, control; ▲, 10 μg/ml; ■, 100 μg/ml; ○, 500 μg/ml; △, 1000 μg/ml. Each point represents the mean of three independent experiments.

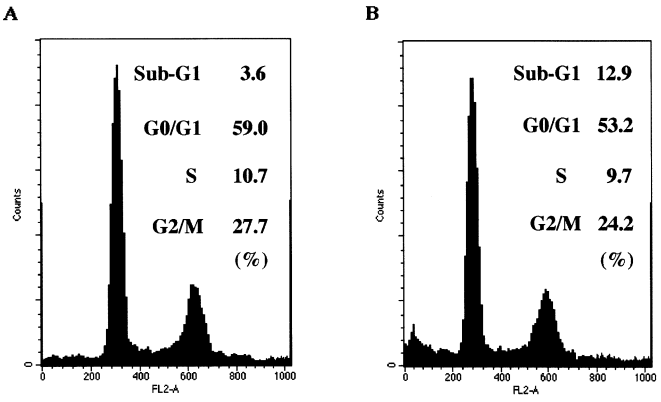

Figure 2. Flow cytometry of HCT116 cells without (A) and with exposure to 500 μg/ml PSK for 96 h (B). The four values in each column represent the percentages of sub-G1, G0/G1, S, and G2/M phase.

TCCTAAGCCCAGATA-3') and antisense (5'-GCAACTTT CTCATATGGTACAGA-3') primers for *T54*, the sense (5'-GACATGGCAGCAGTGCAGAG-3') and antisense (5'-CAG GGCTGAAGGTACCACAC-3') primers for transgelin (*TAGLN*), the sense (5'-GCCAAACATTCCCAACCTAT CC-3') and antisense (5'-CTAGGACACATCAAGACCTG CT-3') primers for *Pirin*, the sense (5'-GGGACCCTGCGCA TGAACCTG-3') and antisense (5'-AGGCAAGTCCAGCA TCTCTGG-3') primers for multidrug resistance protein 3 (*MRP3*, also known as ATP-binding cassette, sub-family C, member 3, *ABCC3*), the sense (5'-TTACACATCAGTC ACAAGTTACAG-3') and antisense (5'-GCATAACAT GCATGATAACTAGCG-3') primers for the lymphotactin (*Lptn*) receptor gene *XCR-1*, the sense (5'-CAGCAACGGA CAGATCTGCA-3') and antisense (5'-GAACCAACAGTC GTCAACATC-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 in the expression pattern of integrin was observed in either cell line. *T54* mRNA expression increased at 12 h in HCT116 cells, while it did not show any significant alterations during a time course analysis in SW480 cells. *Transgelin* mRNA expression

Table I. Representative differential gene expression defined by a 4-fold or greater change in signal intersity, induced by 500 μg/ml PSK treatment for 96 h in the HCT 116 cell line.

Genebank #	Definition	Spot intensity		Genebank #	Definition	Spot intensity	
		Control	PSK treated			Control	PSK treated
NM_003786	**ATP-binding cassette, sub-family C (CFTR/MRP), member 3**	4	33	NM_003570	Cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMP-N-acetylneuraminate monooxyaenase)	54	255
NM_002995	**Small inducible cytokine-subfamily C, member 1 (lymphotactin, Lptn)**	12	93	M32977	Vascular endothelial growth factor	19	89
X07979	**Integrin, ß1 (fibronectin receptor, ß polypeptide, antigen CD29 includes MDF2, MSK12)**	10	54	NM_00830	Glutamate receptor, ionotropic, kainite 1	19	89
NM_003168	Suppressor of Ty (S, cerevisiae) 4 homolog 1	13	72	NM_006577	ß1,3-N acetylglucosaminyltransferase	7	29
NM_012206	hepatitis A virus cellular receptor 1	5	26				
NM_015698	**T54 protein**	30	0	NM_016209	Unknown	25	5
NM_003186	**transgelin (TAGLN)**	37	1	NM_016930	Syntaxin 18	15	3
NM_003662	**Pirin**	17	1	NM_005983	S-phase kinase-associated protein 2 (p45)	15	3
NM_003905	Amyloid ß precursor protein-binding protein 1, 59kD	26	2	NM_014449	Protein 'A'	107	22
D 11086	Interleukin 2 receptor, gamma (severe combined immunodeficiency)	13	1	NM_001640	N-acylaminoacyl-peptide hydrolase	41	9
NM_014481	Apurinic/apyrimidinic endonuclease (APEXnuclease)-like 2 protein	36	3	NM_001168	Baculoviral 1AP repeat-containing 5 (surviving)	108	24
NM_014463	Lsm 3 protein	156	15	NM_15955	CGI-27 protein	18	4
NM_003518	H2B histone family, member A	18	2	U12597	TNF receptor-associated factor 2	18	4
NM_014564	LIM homeobox protein 3	17	2	U05340	CDC 20 (cell division cycle 20, S. cerevisiae, homolog)	218	51
NM_004655	Axin 2 (conductin, axil)	15	2	NM_003258	Thymidine kinase 1, soluble	81	19
NM_004219	Pituitary tumor-transfoming 1	43	6	NM_005342	Insulin induced gene 1	30	7
NM_004153	Origin recognition complex, subunit 1 (yeast homolog)-like	36	5	NM_003451	Zinc finger protein 177	30	7
NM_016359	Clone HQ0310 PRO0310p1	14	2	NM_004260	RecQ protein-like 4	26	6
NM_001034	Ribonucleotide reductase M2 polypeptide	303	49	NM_006461	Mitotic spindle coiled-coil related protein	17	4
NM_015932	Hypothetical protein	24	4	NM_014977	KIAA0670 protein/acinus	25	6
NM_003504	CDC45 (cell division cycle 45, S. cerevisiae, homolog-like	35	6	NM_006467	Polymerase (RNA) III (DNA directed) (23 kD)	41	10
NM_007317	Kinesin-like 4	45	8	NM_004941	DEAD/H (Asp-Glu Ala-Asp/His) box polypeptide 8 (RNA helicase)	28	7
NM_004308	Rho GTPase activating protein 1	43	8	NM_001211	Budding uninhibited by benzimidazoles 1 (yeast homolog), ß	20	5

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.

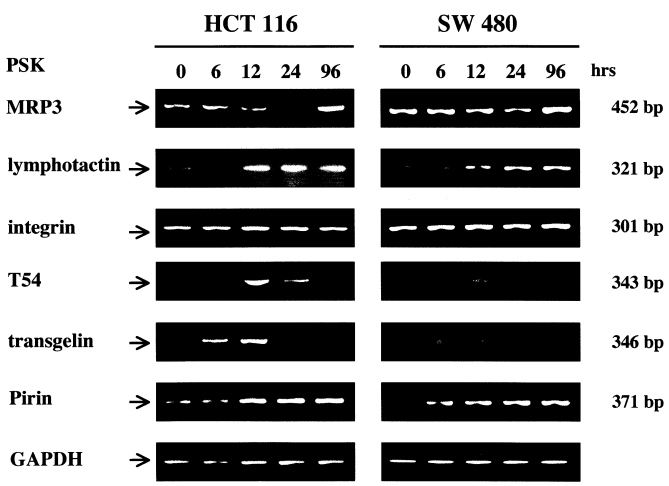

Figure 3. RT-PCR analysis of MRP3, Lptn, integrin ß1, T54, TAGLN, and Pirin mRNA expression by PSK in HCT116 and SW480 cell lines. The amplification products had the expected sizes of 452 bp for MRP3, 321 bp for Lptn, 301 bp for integrin, 343 bp for T54, 346 bp for TAGLN, and 371 bp for Pirin. An amplified fragment of GAPDH demonstrated integrity of the isolated RNA and was used for standardization of candidate genes expressions as an internal control.

gradually increased by 24 h, and then decreased in HCT116 cells, whereas it was absent in controls and induced at 6 h in SW480 cells. *Pirin* mRNA expression markedly increased in a time-dependent manner in both cell lines (3.8- and 9.4-fold of the control level at 96 h in HCT116 and SW480 cells, respectively).

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 through 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.

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 cyclo-oxygenase-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

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-fluorocytosine, 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 CD^{4+} and CD^{8+} 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/CCAAT box transcription factor (NFI/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 *Bcl-3*, the anti-apoptotic transcription factor NF-κB 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

The authors are grateful to H. Seki for her technical assistance. This study was supported in part by grant #16591374 from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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