Review

Antimetastatic Effects of PSK (Krestin), a Protein-bound Polysaccharide Obtained from Basidiomycetes: An Overview

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Abstract

PSK, a protein-bound polysaccharide obtained from cultured mycelia of *Coriolus versicolor* in basidiomycetes, is a biological response modifier, diverse operations of which include an antitumor action. We have previously reviewed recent research which had demonstrated that in animals, PSK has a preventive effect on chemical carcinogen-induced, radiation-induced, and spontaneously developed carcinogenesis (Kobayashi *et al.*, Cancer Epidemiol., Biomarkers & Prev., *2:* 271–276, 1993). We now focus on the effects of PSK once the progression of carcinogenesis has begun, and review what is now known of the preventive action of PSK on cancer metastasis.

Recent research reports that PSK suppresses pulmonary metastasis of methylcholanthrene-induced sarcomas, human prostate cancer DU145M, and lymphatic metastasis of mouse leukemia P388, and that it has prolonged the survival period in spontaneous metastasis models. PSK also suppresses the metastasis of rat hepatoma AH60C, mouse colon cancer colon 26, and mouse leukemia RL male 1 in artificial metastasis models.

PSK influences the steps of cancer metastasis in a number of ways: (*a*) by suppression of intravasation through the inhibition of tumor invasion, adhesion and production of cell matrix-degrading enzymes; (*b*) by suppression of tumor cell attachment to endothelial cells through the inhibition of tumor cell-induced platelet aggregation; (*c*) by suppression of tumor cell migration after extravasation through the inhibition of tumor cell motility; and (*d*) by suppression of tumor growth after extravasation through the inhibition of angiogenesis, the modulation of cytokine production, and the augmentation of effector cell functions. In addition, PSK has suppressed the malignant progression of mouse tumor cells through superoxide trapping.

It therefore seems that PSK suppresses cancer metastasis at any number of different steps rather than at one particular step, and that its primary action mechanism can be ascribed to direct action on the tumor cell as well as to immunomodulation. Since PSK has few side effects and can be administered p.o. over long periods of time, it appears to be a useful agent for controlling or preventing cancer metastasis.

Introduction

Since the acquisition of metastatic properties in tumor cells is one of the most important factors governing clinical prognosis of the individual during the process of carcinogenesis, we therefore need to elucidate the molecular mechanisms of cancer metastasis, as well as to develop effective means to prevent or treat metastasis.

PSK (Krestin), a protein-bound polysaccharide obtained from the *Coriolus versicolor* strain CM-101 belonging to the basidiomycetes, is a biological response modifier capable of exhibiting diverse biological activity (1). The mean molecular weight of PSK is about 9.4×10^4, and its major sugar moiety is a glucan having a main chain β1–4 bond and a side chain β1–3 as well as β1–6 bond that binds to a protein moiety through *O*- or *N*-glycoside bonds (1). Randomized clinical controlled studies have revealed that in combination with chemotherapy, p.o. administration of PSK significantly prolongs the survival period of patients with postoperative gastric and colorectal cancer, as well as those with small cell carcinoma of the lungs (2–6). This agent is now used clinically for the treatment of cancer in Japan.

We have previously reviewed recent research which has demonstrated that PSK in animals exhibits preventive activity against chemical carcinogen-induced, radiation-induced, and spontaneously developed carcinogenesis, and that its mode of action may be antiteratogenic, attributable to radical trapping and to its ability to prevent chromosome injury, as well as to being able to exert immunomodulative effects which can be attributed to the modulation of cytokine production and effector cell function (7). We here focus on the steps or stages of cancer progression, *i.e.*, the multiple process of carcinogenesis, and we review that which has been discovered about the characteristics of PSK as an antimetastatic agent.

Inhibitory Effect of PSK on Metastasis

PSK is effective when used in spontaneous metastasis models as well as in the artificial metastasis models which are described below (Table 1).

Inhibitory Effect of PSK on Spontaneous Metastasis. Tumors produced by the injection of chemical carcinogens often metastasize to other remote organs and provide an appropriate model of spontaneous metastasis. Hosakawa *et al.* (8, 9) developed an experimental model for pulmonary

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Table 1 Summary of antimetastatic effect of PSK

System	Metastatic organ	Animal/tumor	Inoculation site	Effects of PSK administration
Spontaneous metastasis	Lung	C57BL mouse/MCA-induced autochthonous tumor	i.m. (MCA)	Prolongation of survival period (8–10)
	Lung	Athymic nude mouse/human prostate cancer DU-145M and PC-3M	s.c.	Suppression of pulmonary lesions and prolongation of survival period (11)
	Lung	C57BL mouse/mouse melanoma B16-BL6	Footpad	Prolongation of survival period[a]
	Lung	Donryu rat/rat Sato lung cancer	i.m.	Prolongation of survival period (14)
	Liver	Athymic nude mouse/human lung cancer AOI	i.d.	Suppression of liver lesions (12)
	Lymph nodes	CDF_1 mouse/mouse leukemia P388	Footpad	Prolongation of survival period (13)
	Lymph nodes	C3H/He mice/mouse hepatoma MH134	Footpad	Suppression of lymph node lesions (15)
Artificial metastasis	Lung	C57BL mouse/mouse melanoma B16-BL6	i.v.	Suppression of pulmonary lesions[a]
	Liver	Donryu rat/rat hepatoma AH60C	i.v.	Suppression of liver lesions (28)
	Liver	CDF_1 mouse/mouse leukemia RL male 1	i.v.	Suppression of liver lesions (29)
	Liver	CDF_1 mouse/mouse colon 26	i.v.	Suppression of liver lesions and prolongation of survival period (30, 31)

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metastasis by injecting methylcholanthrene into the hindlimb of C57BL/6 mice to produce metastatic autochthonous tumors. Tumors were palpable in mice at the site of methylcholanthrene injection within 12–18 weeks. When the primary tumors were removed surgically after the tumor size had reached 8 mm in diameter, approximately 42% of the mice died of pulmonary metastasis, while the remaining 55% died after local recurrence. They used this model to examine the preventive and therapeutic effects of PSK on the metastasis (8–10). The mean postsurgical survival period of the mice with pulmonary metastasis given PSK i.p. prior to or after surgery was prolonged (56.7–65.0 days) when compared with that of the mice in the surgery-alone group (47.0 days). Statistical analysis by the generalized Wilcoxon test revealed a significant difference of $P < 0.001$ between the surgery alone group and groups given 300 mg/kg of PSK before, or 1 or 8 days after, surgery. They attributed the life prolongation effect of PSK to the decrease in metastatic mortality in the early stages following surgery.

Some kinds of transplantable tumors metastasize to remote organs such as the lung and liver when they are inoculated i.d. or s.c. Mickey *et al.* (11) inoculated hormone-independent human prostatic tumor cell lines into T-cell-defective nude mice and examined the effects of PSK on metastasis. When the highly metastatic cell line DU-145M or PC-3M was inoculated s.c. into nude mice, lung metastasis was observed in every case. The pulmonary metastasis was reduced by the i.p. administration of PSK after the size of primary tumor had reached 500 mm^3 or 1 cm^3. On the other hand, when natural killer cell-deficient beige mice were used instead of nude mice, PSK had practically no effect on pulmonary metastasis, which suggests that natural killer cells are involved in the manifestation of the effect of PSK. Nakatsugawa *et al.* (12) inoculated i.d. the highly metastatic human lung cancer cell line AOI into nude mice and reported enhanced natural killer cell activity and reduction of liver metastasis when PSK was administered p.o. after the primary tumor had reached 5–10 mm size in diameter.

A transplantable tumor inoculated into the footpad of animals metastasized to regional lymph nodes and led finally to metastatic mortality. Tsukagoshi (13) examined the antimetastatic effect of PSK in mice with leukemia P388. PSK was administered i.p. from the day following tumor inoculation for 5 consecutive days. Long-term survivors (>60 days) were observed when this treatment was combined with the removal of primary tumors on the second day after inoculation. Ito *et al.* (14) inoculated Sato lung cancer into the femoral muscle of Donryu rats, administered PSK before and after inoculation, and reported that the survival period was definitely prolonged when combined with the removal of the tumor at 7 days after inoculation. The inhibitory effect of PSK on lymph node metastasis has also been reported by Tsuru *et al.* (15). When the regional lymph nodes of C3H/He mice into whose footpads hepatoma MH134 had been inoculated 17 days earlier were excised and inoculated s.c. into healthy C3H/He mice, the tumors that had metastasized into the lymph nodes grew gradually. The growth of the tumor was significantly suppressed when PSK was inoculated into the lymph nodes 3 days prior to the excision of the primary tumor. They considered that the manifest effect of PSK had been mediated through an enhancement of Lyt 1 and 2 positive T cells in the regional lymph nodes. Other researchers have found that when PSK is combined with such anticancer therapies as chemotherapy and radiation therapy, its antimetastatic effect is enhanced additively or synergistically (16–27).

Inhibitory Effects of PSK on Artificial Metastasis. Artificial metastasis, in which tumor cells are injected directly into an animal blood vessel, is an appropriate model by which to study the metastatic properties of tumor cells after intravasation as well as to evaluate the antimetastatic activity of agents. Intrasplenic injection of some tumor cells produces tumor nodules in the liver via the splenic vein and the portal vein. Morinaga *et al.* (28) examined the effect of PSK on liver metastasis by inoculating hepatoma AH60 into the portal vein of Donryu rats. At 3 weeks after inoculation there were 62.7 metastatic foci in the liver of the untreated

control group and 41.1 in the group given PSK p.o. The administration of PSK thus significantly reduced liver metastasis. They also reported that p.o. administration of PSK significantly reduced both the number of liver metastatic foci and the take rate in a liver metastatic model by inoculating leukemia RL male 1 into the portal vein of CDF_1 mice (29). Takahashi *et al.* (30) have demonstrated the inhibitory effect of PSK in a liver metastatic model by using mouse colon 26 tumor. They found that the mean number of liver metastatic foci 3 weeks after inoculation of the tumor into the portal vein of CDF_1 mice was 125 in the untreated control group and 89.5 in the group given PSK p.o.; this suggests that PSK had significantly reduced the development of liver metastasis (30). Using the same mouse model of hepatic metastasis of a colon tumor, other researchers have confirmed that p.o. administration of PSK significantly reduces the weight of the metastatic liver (31) and significantly prolongs the survival period of mice (32). PSK also inhibits metastasis when used in combination with other anticancer therapies (33–39). Although liver metastasis was aggrevated by the combination of the administration of pyrimidine fluoride derivatives and splenectomy in C57BL/6 mice inoculated with leukemia EL4 intrasplenically, the aggrevatory effect disappeared and the metastasis was reduced by the p.o. administration of PSK (36). Lung metastasis was aggrevated by surgical stress (thoracotomy) in Donryu rats after i.v. inoculation of Sato lung cancer, but it was prevented by the i.p. administration of PSK (37).

Mechanism of Action

The metastasis of tumor cells is a multistage phenomenon, and many factors are involved in the process, from the detachment of the primary lesion to the growth of the metastatic lesion. It appears that the antimetastatic effect of PSK can be attributed not only to the immunomodulatory effect already mentioned (7) but also to such various effects on tumor cells as the inhibition of motility and malignant progression. It is clear, however, that PSK works not at a specific stage of the metastasis process but as a multistep operator (Table 2).

Table 2 Summary of effects of PSK on multistep process of metastasis

Process of metastasis	Effects of PSK
Detachment	Suppression of tumor cell motility *in vitro* and *in vivo* (45)
Intravasation	Inhibition of tumor cell invasion, attachment, haptotactic migration, and productivity of extracellular matrix-degrading enzymes *in vitro*[a]
Intravascular migration and attachment to vessel	Suppression of tumor cell-induced platelet aggregation *in vitro* (40)
Extravasation	Suppression of functions of cytoskeletal proteins *in vitro* (42–44)
Migration	Suppression of tumor cell motility *in vitro* and *in vivo* (45)
Proliferation	Suppression of tumor angiogenesis *in vivo* (46) Immunomodulation *in vitro* and *in vivo* (41, 47–67)
Others	Trapping of active oxygen *in vitro* and *in vivo* in mouse tumor progression model (69–71) Suppression of c-Ha-*ras* oncogene expression and production of basic fibroblast growth factor *in vivo* (72) Enhancement of human leukocyte antigen class II expression in tumor cells *in vitro* (73) Suppression of sister chromatid exchange frequency of bone marrow cells in mice treated with anticancer agent *in vivo* (75)

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Effects of PSK on the Detachment Process from the Primary Lesion. The migration of tumor cells follows the detachment of the tumor cells from the primary lesion, and its success depends on the adhesive properties of the cells. Although no direct evidence relates the action of PSK to the suppression of the adhesive properties of tumor cells, and although PSK seems to have little effect on the expression of the neural cell adhesion molecule in mouse metastatic melanoma B16-BL6 *in vitro*,[2], it does suppress the motility of tumor cells *in vitro* and *in vivo*, as we shall see (40).

Effects of PSK on the Intravasation Process. Detached tumor cells infiltrate the stroma, destroy the basement membrane, and enter the blood vessels. Using an *in vitro* model of invasion in a Transwell chamber system, Matsunaga *et al.*[2] discovered that PSK has a suppressive effect on the chemoinvasion and haptoinvasion of mouse melanoma B16-BL6; on the attachment of the tumor cells to the solubilized basement membrane preparation extracted the Engelbreth-Holm-Swarm mouse sarcoma (Matrigel), fibronectin, laminin, and collagen type IV; and on the chemotactic and haptotactic migration of tumor cells to fibronectin. The effect of PSK is dose dependent, and its main mechanism seems to be some kind of interaction between PSK and the components of basement membrane.

Extracellular matrix-degrading enzymes which are secreted from tumor cells are involved in the process of destroying the basement membrane. Matsunaga *et al.*[2] have demonstrated that PSK has a suppressive effect on the production of collagenase type IV in mouse melanoma B16-BL6 *in vitro*, possibly not by the induction of a tissue inhibitor of metalloprotease. Some cytokines have an effect on the production of these enzymes. Although Abe *et al.* (41) have demonstrated that PSK induces the production of IFN-γ a down-regulator of these enzymes, direct evidence of its effect on extracellular matrix-degrading enzyme production *in vivo* has not been reported.

Effects of PSK on the Process of Intravascular Migration and Attachment to Vessels. Tumor cells in the blood vessels attach to the peripheral capillary bed of some organs. Tanaka et al. (40) have reported that PSK inhibits tumor cell-induced platelet aggregation *in vitro*. They suggest that PSK acts by inhibiting tumor cell attachment to vascular endothelial cells. PSK seems to have little effect on the expression of CD49d, CD49e, CD44, and lymphocyte function associated antigen-1 molecules in metastatic mouse melanoma B16-BL6,[2] which are known to be involved in the adhesion of tumor cells to endothelial cells or the basement membrane. Further, PSK has little suppressive

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effect on the expression of the vascular cell adhesion molecule-1 in human vascular endothelial cells.[2]

Effects of PSK on the Extravasation Process. Tumor cells attached to a vessel secrete enzymes, destroy the basement membrane, and invade the tissues. During this process, tumor cells may become deformed and deformity may have consequences in addition to the destruction of the basement membrane. There are no reports that PSK has any suppressive effect on these phenomena, although the agent inhibits the function of cytoskeletal proteins such as tublin (42–44).

Effects of PSK on the Migration Process after Extravasation. Tumor cells which invade tissue migrate and colonize locally. The motility of tumor cells probably has a role in this process. Katano *et al.* (45) examined the *in vitro* effect of PSK on the motility of tumor cells by the capillary method. The motility of mouse leukemia EL4, Ehrlich carcinoma, and human null cell leukemia Hoon was dose dependently suppressed after incubation of tumor cells with PSK for 12 h. The effect of PSK was dependent on the incubation time, and a maximum inhibition (about 50%) was observed for a 4-h treatment. Tanaka *et al.* (40) also found that PSK suppresses the cell motility of colon 26 tumor. In addition, Katano *et al.* (45) have examined the *in vivo* effect of PSK on tumor cell motility. PSK-treated EL4 cells were less invasive than nontreated cells after the s.c. inoculation of the tumor in the abdominal wall of C57BL/6 mice.

Effects of PSK on the Growing Process in Metastatic Foci. Metastasis is initiated after extravasated tumor cells grow in local tissues. A supply of nutrients from the blood is essential for the progressive growth of the tumor cells, and angiogenesis characteristic of tumor cells has been observed at the tumor site. The inhibitory effect of PSK on tumor-associated angiogenesis may be associated with the growing process of tumor cells after colonization of a metastatic lesion. Kumar *et al.* (46) examined the effect of PSK on angiogenesis in mice by the dorsal air sac method. Millipore chambers enclosing mouse hepatoma MH134 were embedded s.c. in the back skin of mice, and the muscular fascia was observed chronologically. While abundant capillaries rich in the Weibel-Palade, which is characteristic of angiogenesis, had formed in the control group, capillary formation was clearly inhibited in mice to whom PSK had been given. The inhibitory effect of PSK was accompanied by a significant decrease in the alkaline phosphatase activity of skin tissues attached to the chamber.

The host immune system has an important effect on the process of tumor cell growth in metastatic lesions. PSK acts on monocytes, macrophages, and T cells, modulates the production of cytokines, and enhances the antitumor activity of natural killer cells, T cells, lymphokine-activated killer cells, and macrophages. That is, it induces monokines (41, 47–51) and facilitates lymphokine production (41, 52) by human peripheral blood mononuclear cells *in vitro*. p.o. administration of PSK induces gene expression of monokines in mouse spleen cells and human peripheral blood mononuclear cells (51, 53). PSK enhances the function of killer T cells, natural killer cells, and macrophages *in vitro* as well as *in vivo* (54–63). In addition, PSK halts the reduction of cytokine-producing capacities and natural killer cell and macrophage activities that have been induced by tumor inoculation (53, 64–66). The mode of action may be an antagonistic effect on humoral immunosuppressive factors produced in the tumor-bearing host (53, 67).

The effects of PSK on the immune system have been studied by using models in which PSK has been shown to suppress metastasis. Takahashi *et al.* (30) and Suo *et al.* (31) reported that the activity of splenic natural killer cell and lymphokine-activated killer cell as well as interleukin-2-producing capacity were enhanced by the administration of PSK in CDF_1 mice after colon 26 tumor had been inoculated into the portal vein. In a population analysis of liver nonparenchymal cells in rats inoculated with hepatoma KDH-8 in the portal vein, the enhancing effect of PSK on natural killer cell activity was marked in the large granular lymphocyte-rich cell fraction. Morinaga *et al.* (29) and Tani *et al.* (68) reported that the antitumor activity of mouse liver macrophages was enhanced by the p.o. administration of PSK, while Shinomiya *et al.* (36) reported that the numbers of liver Kupffer cells as well as their phagocytic activities were enhanced by the p.o. administration of PSK in mice inoculated with leukemia EL4 intrasplenically. The enhancement of effector cell activity in the liver appears to play an important role in the manifestation of the preventive or therapeutic effect of PSK.

Effects of PSK on the Tumor Progression. Metastatic tumor cells are, in short, both more undifferentiated and more highly malignant than nonmetastatic cells. Okada *et al.* (69) developed a progression model of mouse tumor cells adapted to acquire malignancy. They established a mouse cell line QR-32 that is weakly tumorigenic when injected s.c. into a syngeneic host in single cell suspensions but shows a high incidence of lethal growth when implanted s.c. with a gelatin sponge. They then examined the effect of PSK on the malignant changes in this tumor (70, 71). Only 50% of the QR-32 tumor cells in the PSK-administered group acquired tumorigenecity, which was significantly lower than the percentage for the control group which was 100% ($P < 0.05$). Since host cells reactive to the gelatin sponge release active oxygen that leads to malignant changes in QR-32 cells (69), PSK seems to increase the level of manganese superoxide dismutase, copper superoxide dismutase, catalase, and glutathione peroxidase locally at the site of the tumor tissue; this results in the suppression of oxygen radicals and the prevention of the malignancy.

The mechanism of malignant changes in tumor cells has been examined at a molecular level from the standpoint of growth factors and oncogenes. Mickey (72) reported that both c-Ha-*ras* proto-oncogene expression and the production of basic fibroblast growth factors in highly metastatic rat MAT-LyLu tumor cells were suppressed by the i.p. administration of PSK in Copenhagen rats with the tumor. Araya *et al.* (73) found that the expression of human leukocyte antigen class II was enhanced by *in vitro* PSK in human gastric carcinoma KATO-3, resulting in an increase of sensitivity to cytotoxicity of interleukin-2-activated killer cells acting against the tumor cells. Hirose *et al.* (74) reported that some kind of messenger RNA transcription in rat hepatoma AH66 was induced or suppressed by *in vivo* administration of PSK. Hasegawa *et al.* (75) found that the administration of PSK significantly suppressed the increased frequency of sister chromatid exchange in bone marrow cells of C57BL/6 mice treated with mitomycin C. However, no evidence of a direct effect of PSK on the deletion of suppressor genes has been reported.

Clinical Studies

Several investigators have analyzed the results of controlled randomized clinical studies to examine the effects of PSK in the prevention of metastasis or recurrence, although none of these reports evaluates it directly. Mitomi *et al.* (5, 76) studied the clinical efficacy of PSK following curative resection of colorectal cancer by a randomized controlled study. Their study included 462 patients who were registered at 35 facilities in Kanagawa Prefecture in Japan between March 1985 and February 1987. Of these 462 patients, 448 (97.0%) were evaluated. The control group consisted of patients who received i.v. injection of mitomycin C (6 mg/mm^2) on the day of surgery and on the following day and p.o. doses of 5-fluorouracil (200 mg/day) for more than 6 months starting during the second or third postoperative week. The PSK treatment group consisted of patients who had received p.o. doses of PSK (3 g/day) for more than 3 years, in addition to the chemotherapies used for the control group. When 449 patients were followed up for 5 years after surgery, both the disease-free survival period and whole survival period were significantly longer in the PSK treatment group than in the control group ($P = 0.0214$ and $P = 0.0272$, respectively; general Wilcoxon test). Mitomi *et al.* (5) subsequently analyzed the influence of PSK on tumor recurrence. Tumor recurrence, as judged on the basis of a general evaluation of physical examinations, X-ray, computed tomography, ultrasonography, and laboratory findings, was noted in 143 patients during the 5-year period (83 patients in the control group and 60 in the PSK treatment group). An analysis of the hazards of recurrence, which was carried out every 3 months after surgery, revealed that the recurrence had a peak between the 9th and 12th month after surgery in both groups, and that the peak incidence of recurrence in the control group was about 1.7 times as high as that in the PSK treatment group. When the first recurrence was divided into four types (local recurrence, systemic metastasis such as liver and lung, peritoneal dissemination, and lymph nodes metastasis), the rate of tumor recurrence in the PSK treatment group was lower than that in the control group in all patterns of recurrence, and the intergroup difference in the incidence of lymph node metastasis was significant ($P < 0.05$). Takashima *et al.* (77) compared the therapeutic effects between two groups of patients with advanced colorectal cancer who underwent curative resection: a control group (71 patients who received an i.v. mitomycin C injection on the day of surgery and on the following day and received tegafur suppositories for 1 year starting during the second postoperative week); and a PSK treatment group (53 patients who received p.o. PSK doses, 3 g/day, for 1 year in addition to the therapies used for the control group). This comparison revealed a higher survival rate and a lower incidence of recurrence during the 2-year period among the PSK treatment group. Nakazato *et al.* (3), Kondo *et al.* (78), and Torisu *et al.* (79) reported that disease-free survival after curative resection of gastric or colorectal cancer was significantly prolonged by p.o. PSK.

As for the treatment of metastatic lesions themselves, a number of studies report that patients have responded positively to treatment when PSK was used alone or when PSK was combined with other therapies (80–85). Furthermore, a randomized controlled study of immunochemotherapy after surgical treatment of gastric cancer revealed that the survival period of patients with lymph node metastasis was significantly prolonged by PSK treatment (86).

Summary

PSK is currently used as an immunotherapeutic agent for gastric, colorectal, and lung cancers in Japan. It has virtually no adverse effects, and it can be administered p.o. over a longterm. Consequently, its use need not be limited to the treatment of cancer, and, as our previous paper suggested, it should in the future prove valuable as a general chemopreventive agent and, as this review shows, as an antimetastatic agent. The principal mechanisms of PSK may act as an inhibitor of the motility, invasion, and progression of tumor cells, in addition to its role as an immunomodulator.

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