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Immunochemotherapy benefits in gastric cancer patients stratified by programmed death-1 ligand-1

Jun-Te Hsu, MD,[a,1] *Chih-Sin Hsu, PhD,*[b,1] *Puo-Hsien Le, MD,*[b] *Tse-Ching Chen, MD, PhD,*[c] *Wen-Chi Chou, MD,*[d] *Chun-Yen Lin, MD, PhD,*[b,*] *and Ta-Sen Yeh, MD, PhD*[a,*]

[a] *Department of Surgery, Chang Gung Memorial Hospital at Linkou, Chang Gung University, College of Medicine, Taoyuan City, Taiwan*

[b] *Department of Gastroenterology, Chang Gung Memorial Hospital at Linkou, Chang Gung University, College of Medicine, Taoyuan City, Taiwan*

[c] *Department of Pathology, Chang Gung Memorial Hospital at Linkou, Chang Gung University, College of Medicine, Taoyuan City, Taiwan*

[d] *Department of Medical Oncology, Chang Gung Memorial Hospital at Linkou, Chang Gung University, College of Medicine, Taoyuan City, Taiwan*

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ABSTRACT

Background: Effectiveness of protein-bound polysaccharide K (PSK) during adjuvant chemotherapy in gastric cancer patients expressing programmed death-1 ligand 1 (PD-L1) has not been investigated. Investigating this might help in triaging candidates eligible to immunochemotherapy.

Materials and methods: In total, 918 patients with stages II and III gastric cancer, undergoing curative gastrectomy, and receiving adjuvant chemotherapy were enrolled in a prospective database, and the patients were retrospectively reviewed. We classified those patients into four cohorts stratified by PD-L1 expression and PSK administration, namely PD-L1, PSK (−,+); PD-L1, PSK (−,−); PD-L1, PSK (+,+); and PD-L1, PSK (+,−). In addition, another independent cohort of 20 patients undergoing radical gastrectomy was prospectively recruited to check their immunological cells of sera before and 2 mo after PSK administration.

Results: PSK treatment was an independent prognostic factor for patient's overall survival ($P = 0.020$), whereas PD-L1 expression per se was not. Administration of PSK prolonged patient survival in stages IIIA and IIIB ($P = 0.031$) but not in stage II or stage IIIC. Patients with negative expression of PD-L1, treated with PSK had longer survival than those not treated with PSK ($P = 0.033$). PSK did not affect the survival of patients with positive expression of PD-L1, ($P = 0.421$). The percentages of natural killer and natural killer T (NKT) cells, but not Th1, Th17, Treg, or IFN-γ+/CD8+ T cells, were significantly increased in PD-L1 (−) patients treated with PSK. However, these findings were not evident in PD-L1 (+) patients.

Conclusions: PSK treatment preferentially confers a survival gain for patients with stage IIIA/IIIB gastric cancer, especially in the PD-L1 (−) subpopulation.

* *Corresponding author*. Department of Surgery, Chang Gung Memorial Hospital, 5 Fu-Hsing Street, Kwei-Shan District, Taoyuan City, Taiwan. Tel.: +886 3 3281200x3219; fax: +886 3 3285818.

E-mail addresses: chunyenlin@gmail.com (C.-Y. Lin), tsy471027@adm.cgmh.org.tw (T.-S. Yeh).

[1] Both had an equal contribution.

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Introduction

Gastric cancer remains the fourth most common malignancy and the second leading cause of cancer-related deaths worldwide.[1] Accumulated milestone evidence indicates that radical gastrectomy plus adjuvant 5-fluorouracil (5-FU)–based chemotherapy prolong the patients' survival. However, long-term results from patients with nodal metastasis are still disappointing.[2–5] Therefore, multidisciplinary therapeutic strategies other than conventional surgery, chemotherapy, and radiation are urgently needed for gastric cancer patients.

Immunotherapy provides another facet of breakthrough to overcome the constraints of current management. Multiple mechanisms that allow tumors to escape rejection by the immune system have been identified, including downregulation of human leukocyte antigen (HLA) class 1, production of immunosuppressive cytokines such as transforming growth factor beta 1, interleukin-10, and interleukin-6, and infiltration of regulatory T cells (Treg) and bone marrow–derived stem cells.[6] Recently, immune checkpoints are recognized as inhibitory pathways hardwired into the cancer immune microenvironment, which are responsible for cancer evasion and potential failure of various kinds of immunotherapy.[7] Cytotoxic T-lymphocyte–associated antigen-4 and programmed cell death-1 (PD-1) receptors are two coinhibitory receptors relevant to those immune checkpoints. PD-1 (also known as B7-H1 or CD274) is highly expressed on tumor-infiltrating lymphocytes and, when engaged by its ligands, serves to inhibit T-lymphocyte activity. Programmed death-1 ligand-1 (PD-L1) is expressed on many solid tumors, including melanoma, ovarian, lung, renal, and gastric carcinoma, and is largely regarded as a biomarker and/or target of immunotherapy.[8]

Protein-bound polysaccharide K (PSK) (KRESTIN; Kureha Chemical Industry Co, Tokyo, Japan) is one of the most common medicinal mushroom extracts used as an immune modulator and an oral biological response modifier in cancer therapy in Japan.[9] Randomized controlled studies have proven that combined use of PSK with chemotherapeutic agents significantly prolongs the overall survival (OS) of patients with gastric cancer, colorectal cancer, and lung cancer, importantly without severe adverse drug reactions.[10–13] Various mechanisms of augmented antitumor action have been suggested for PSK, including activation of natural killer (NK) cells, inhibition of immunosuppressive cytokines production, upregulation of human leukocyte antigen class 1 expression on cancer cells, and reduction of T-cell apoptosis through inhibition of nuclear factor kappa B.[9] Nevertheless, the effect of PD-L1 on the effectiveness of PSK as an immune modulator has not been investigated.

The aim of this study was to analyze the long-term outcome obtained in gastric cancer patients who underwent radical surgery and received adjuvant immunochemotherapy (5-FU–based regimens plus PSK). Those patients were stratified by PD-L1 expression in one of the largest relevant surgical centers in the world. Also, to explore the effects of PSK on host immune cells, we conducted a prospective study to determine the changes in patients' peripheral blood immune cells according to the status of PD-L1 expression before and after PSK administration, while receiving adjuvant chemotherapy.

Patients and methods

Patient treatments and characteristics

The study was approved by the Institutional Review Board of Chang Gung Memorial Hospital. In total, 918 patients with stage II-III (based on AJCC seventh edition) gastric cancer undergoing radical gastrectomy and receiving adjuvant chemotherapy in Chang Gung Memorial Hospital from 2000 to 2011 were enrolled in a prospective database, and the patients were retrospectively reviewed. Among them, 560 patients received adjuvant chemotherapy alone, symbolized as PSK (–), and 358 received adjuvant chemotherapy plus PSK, symbolized as PSK (+). In our institute, adjuvant chemotherapy regimen (predominately either 5-FU intravenously or tegafur/uracil orally) was used to treat gastric cancer patients, usually up to 6 mo. PSK, 3-g daily (1g tid), was continuously given for 6 to 12 mo (median, 11.7 mo) once adjuvant chemotherapy (usually 6-8 wk after radical gastrectomy) was commenced. Clinicopathologic parameters such as the patients' demographics, tumor location and stage, operation type, pathologic findings, and long-term survival were obtained from the prospectively constructed database for gastric cancer. In addition, the status of PD-L1 expression in gastric cancer tissue was determined using immunohistochemistry. Those with high expression of PD-L1 were abbreviated as PD-L1 (+), whereas those with low expression of PD-L1 were abbreviated as PD-L1 (–). Accordingly, we further divided the 918 patients into four cohorts stratified by PD-L1 expression and PSK administration, namely PD-L1, PSK (–,+); PD-L1, PSK (–,–); PD-L1, PSK (+,+); and PD-L1, PSK (+,–). The survival duration was calculated either as the time from surgery to cancer-related death or as the time since the date of the last follow-up (December 31, 2012). The median follow-up time was 31.6 mo. In addition, a cohort of 20 gastric cancer patients undergoing radical gastrectomy was prospectively recruited since June 2013. Their immunologic cells were investigated before and 2 mo after PSK administration, while receiving adjuvant chemotherapy.

Tissue microarrays and immunohistochemical investigation of PD-L1

Formalin-fixed and paraffin-embedded tissue samples were arrayed using an automated tissue-arraying instrument (BEECHER ATA-27, Beecher Instruments, Sun Prairie, WI). Three representative areas of each tumor were selected and marked on the tissue slide on which hematoxylin-eosin staining was performed, and the corresponding tissue block for each tumor was sampled. The designated zone from each donor block was punched with a tissue cylinder of 1-mm diameter, and the sample was transferred to a recipient block. The arrayed tissue sections were used for immunohistochemical investigation of PD-L1 (1:100, Abcam, ab58810, United Kingdom). Tissue sections of 3-μm thickness were deparaffinized in xylene and rehydrated in a graded ethanol series. The immunohistochemical study was performed using an automated immunostainer (BOND-MAX, Leica, Wetzlar,