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Anticancer Effect of *Phellinus linteus*; Potential Clinical Application in Treating Pancreatic Ductal Adenocarcinoma

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Abstract

Pancreatic cancer (ductal adenocarcinoma) is one of the most lethal malignancies in gastrointestinal system. Till now, only margin-negative pancreatectomy is known to be the best treatment option for long-term survival. However, the resection rate is reported to be less than 20%. Even in cases of curative resection, most patients generally experience disease recurrence and ultimately die of metastatic disease. Therefore, surgery alone is not enough and adjuvant systemic chemotherapy should be considered for proper management of pancreatic cancer. But, its related toxicity and insufficient oncologic effect requires development of less toxic and more effective alternative treatments for pancreatic cancer. *Phellinus linteus* (PL), a basidiomycete, is a species of mushroom, which is reported to have the most potent antitumor effect of the basidiomycetes. Recently, there are accumulating researches discovering biologic mechanisms of PL in antitumor effect. However, very few studies were done in pancreatic cancer treatment. In this review, literatures demonstrating the anticancer effects of PL were summarized and some encouraging research data suggesting mushroom component can be alternative approach even in treating pancreatic cancer were reviewed, including our preliminary data suggesting the potential clinical application of PL in treating pancreatic cancer.

Keywords: Pancreatic cancer; *Phellinus linteus*; Basidiomycete; Adjuvant chemotherapy

Introduction

Pancreatic cancer (ductal adenocarcinoma) is one of the most lethal gastrointestinal malignancies. Complete surgical resection is a basic requirement for long-term survival. However, the resection rate of pancreatic cancer is only 15-20% because the disease is locally advanced or metastatic at initial diagnosis. Even in cases of curative resection, most patients experience disease recurrence, especially in the liver, and ultimately die of metastatic disease. Gemcitabine has been used as standard chemotherapy for pancreatic cancer [1], but, most clinical trials that have tested combinations with other cytotoxic agents have failed to demonstrate markedly improved oncologic outcomes [2]. In spite of high morbidity, FOLFIRINOX is an emerging chemotherapeutic agent for metastatic pancreatic cancer treatment [3]. However, studies are needed to determine the appropriate dosages and clinical applications, such as use in postoperative adjuvant or neoadjuvant therapy. Therefore development of less toxic and effective alternative treatments for pancreatic cancer is also needed. A potential alternative strategy is to investigate and understand the mechanism of action of natural medicines with anticancer effects.

Phellinus linteus (PL), a basidiomycete, is a species of mushroom whose fruiting body is called sanghuang (Figure 1). This mushroom has been widely used in ancient herbal medicines in Korea, China, and Japan. In 1968, the antitumor activity of polysaccharides from PL was first reported [4]. PL is reported to have the most potent antitumor effect of the basidiomycetes [5]. In the last few decades, the use of PL for suppressing cancer or enhancing immunity [6] has been actively investigated. Biological effects were found to be associated with isolated polysaccharides, proteoglycans and other organic compounds. Isolated compounds and complex extracts from PL inhibit signal pathways in various cancer cells. However, few clinical studies to date have tested the oncologic effects of PL, and only a few cases have demonstrated the therapeutic effect of PL on primary liver cancer. In Japan, a patient with hepatocellular carcinoma and multiple lung metastases was reported to experience complete tumor regression 6 months after treatment with PL extract [7]. Radiation therapy in combination with PL ingestion resulted in spontaneous remission of hepatocellular carcinoma with skull metastasis in a Korean patient [8].

In this review, we summarize several studies that show the anticancer effects of PL. We also provide our preliminary data that suggests the potential clinical application of PL in treating pancreatic cancer.

Figure 1: General appearance of *Phellinus linteus*.

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Anticancer effects of *Phellinus linteus*

The biologically active compoundsin PL are reported to be polysaccharides [9], acidic proteoglycans with mixed α- and β-linkages, and a (1→6)-branched type (1→3)-glycan [10]. In 1969, Chihara et al. [11] demonstrated that polysaccharide extracts from the basidiomycete fungus PL suppressed tumor growth in vivo. Recently, new furopyranone compounds, phellifuropyranone A and meshimakobnol A/B, isolated from fruiting bodies of wild PL, demonstrated antiproliferative activity against mouse melanoma and human lung cancer cells in vitro [12]. However, the molecular mechanisms responsible for the direct antitumor activity have not been fully investigated.

Protein-bound polysaccharides isolated from PL suppressed the proliferation and colony formation of colon cancer cells (SW480). This effect was found to be mediated by cell cycle arrest at G2/M phase and associated with down regulation of cyclin B1 [13]. Isolated low molecular weight compounds from PL such as hispolon demonstrated a dose-dependent inhibition of human epidermoid KB cell proliferation [14]. Several recent studies demonstrated potential mechanisms of the direct anticancer effects of PL extracts on tumor proliferation, growth and invasiveness (Table 1).

Immunomodulation

Polysaccharides and proteoglycans extracted from PL might stimulate the proliferation of T lymphocytes and the humoral immune function, resulting in a secretory and cellular macrophage response. Nonspecific immune functions mediated by natural killer cells and macrophages increased in vivo and in vitro with treatment with PL. PL stimulated humoral immune functions such as T-dependent and -independent primary antibody responses, and acted as a polyclonal activator of B cells. PL exhibited a wider range of immunostimulation and antitumor activity than other polysaccharides isolated from other basidiomycetes [15].

Kim et al. [16] demonstrated that a novel polysaccharide-protein complex extracted from PL markedly increased B-cell proliferation, production of cytokines and nitric oxide from macrophages, and NK cell-mediated killing of YAC-1 lymphoma cells in vitro. These results suggest that PL is a biological response modifier. In vivo, when HT-29 colon cancer cell-bearing mice were treated with proteoglycans extracted from PL, a relative increase in spleen and thymus weights were noted. Significant changes in plasma biochemical parameters showed that PL acted as an immunopotentiator partly through protecting T cells and enhancing mucosal IgA responses [17]. In human hepatoma (Hep3B) cell-transplanted mice, an extract of a mycelial culture from PL induced significant reduction of tumor size and increased T cell numbers, and increased IL-2, IFN-γ, and TNF-α secretion and NK cell activity and phagocytic ability [18,19]. NK cells are especially known for their ability to lyse a variety of tumor cells by exocytosis of perforin-containing granules, subsequently forming lytic pores by perforin on the target cell membrane. In addition, NK cells are responsible for early inhibitory effects on tumor growth and for long-term tumor-suppressive effects [20,21].The importance of innate immunity in pancreatic cancer was recently reiterated by the strong correlation between the absolute numbers of NK cells circulating in patients before chemotherapy and survival and progression-free interval. The average percentage of NK cells before therapy positively correlated with NK cytotoxic lytic units, suggesting that cytotoxic functions of NK cells are important in pancreatic cancer [22].This suggests that PL-induced immunomodulation, especially regarding NK cell activity, needs to be further evaluated in treating cancer patients.

Inhibition of Metastasis

Chemotherapy is an important therapeutic modality for managing a variety of cancers. However, chemotherapy frequently fails to achieve a satisfactory therapeutic outcome such as complete remission or prevention of distant metastasis with no major chemotherapy-related side effects [23]. Cancer metastasis, in particular, is a major medical problem in treating cancer.

The potential application of PL as an immunotherapeutic agent, especially for cancer metastasis, has been actively investigated. PL alone significantly prolonged the survival rate of B16F10 cell-implanted mice, inhibited tumor growth in NCI-H [23] cell-implanted nude

Author, year	Direct anticancer effect	Observation	Mechanism
Song et al. [56]	Inhibits tumor growth and invasion (colon cancer cells)	-PL significantly inhibited cell proliferation and decreased β-catenin expression in SW480 cells. -Expression of cyclin D1, TCF/LEF, MMP-9 were also significantly reduced by PL treatment.	Inhibition of Wnt/β-catenin signaling
Li et al. [17]	Antiproliferative effect (colon cancer cells)	-PL inhibited HT-29 cell proliferation -Decreased expression of Reg IV, and EGFR mRNA - Decreased plasma Reg IV, EGFR, Akt concentration	Down-regulating Reg IV and EGFR → may lead to disruption of the Reg IV/EGFR/Akt signaling pathway
Lu et al. [57]	Inhibits tumor growth (breast and bladder cancer cells)	-Hispolon from PL inhibited cancer cell growth - p21WAF1, a cyclin-dependent kinase inhibitor, was elevated in hispolon-treated cells - MDM2, a negative regulator of p21WAF1, was degraded after hispolon treatment. -Activated ERK1/2 was recruited to MDM2 leading to MDM2 degradation	Hispolon from PL ubiquitinates and downregulates MDM2 via MDM2-recruited activated ERK1/2 and upregulates p21WAF1
Guo et al. [31]	Cancer growth arrest (lung cancer cells)	-PL induces cell-cycle arrest at a low concentration. -Associated with decrease of CDK 2, 4 and 6	PL suppresses the activation of CDK → unable to form complexes with cyclin D,E, or A → maintains phospho-Rb → blockage of cell-cycle progression
Sliva et al. [36]	Suppressed growth and invasive behavior (breast cancer cells)	-PL inhibits proliferation and colony formation, -Growth inhibition is mediated by cell cycle arrest at S phase through upregulation of p27Kip1 - Suppression of invasion by inhibition of cell adhesion, cell migration and cell invasion through suppression of uPA secretion	Inhibition of serine-threonine kinase AKT signaling, upregulation of p27Kip1, and suppression of uPA

TCF/LEF, T-cell factor/lymphocyte enhancer binding factor; MMP, matrix metalloprotease; EGFR, epidermal growth factor receptor; ERK1/2, extracellular signal-regulated kinase1/2; CDK, cyclin-dependent kinase; uPA urokinase-plasminogen activator

Table 1: Direct anticancer effects of PL and proposed molecular mechanisms.

mice, and reduced the frequency of pulmonary metastasis of B16F10 cell melanomas compared to adriamycin, which significantly inhibited tumor growth, but only slightly inhibited metastasis. PL was not directly toxic to cancer cells, suggesting its mechanism is through stimulation of the immune response [24]. In another study [25], PL markedly inhibited melanoma cell metastasis in mice by a direct inhibitory effect on the adhesion to and invasion through the extracellular matrix of cancer cells. In addition, PL increased macrophage NO production. Lee et al. [26] demonstrated that PL might inhibit metastasis at least in part through the regulation of urokinase type plasminogen activator (uPA) associated with tumor cell-induced platelet aggregation. Although PL showed no cytotoxicity against invasive melanoma cells (B16BL6 cells), PL inhibited platelet aggregation induced by B16BL6 cells. It also disrupted the adhesion to gelatin and invasion of B16BL6 cells in a concentration-dependent manner. PL dose-dependently inhibited pulmonary metastatic colonies up to 55.5% in C57BL/6 mice intravenously injected with B16BL6 cells compared with untreated controls. In addition, PL resulted in concentration-dependent down regulation of the expression of uPA, a key protein in invasion and metastasis of tumor cells.

Recurrence, especially of distant liver metastasis, is a major problem in treating pancreatic cancer following margin-negative pancreatectomy. Recently, FOLFIRINOX successfully demonstrated excellent survival in patients with metastatic pancreatic cancer. However, the significant side effects could limit its general application in clinical practice [3,27,28]. Therefore, effective anti-metastatic therapeutic agents need to be further investigated. PL may be a potential candidate as a natural nontoxic agent.

Induction of Apoptosis

Apoptosis is fundamental process essential for cancer cell death. Deregulation of apoptosis is related to cancer progression. Caspase 3 is important in the propagation of apoptotic signaling. In the caspase cascade, a loss of mitochondrial transmembrane potential results in an increase in the permeability of the mitochondria membrane. Cytochrome C is released to the cytosol, leading to apoptosis [29].

The first description of PL-mediated apoptosis in colon cancer cells was reported in 200413. In this study, polysaccharide from PL (Mesimaw) had an antiproliferative effect on SW480 human colon cancer cells. The effect was mediated through the induction of apoptosis and G2/M cell cycle arrest, and was associated with a decrease in Bcl-2, an increase in the release of cytochrome C, and a reduction in cyclin B1 expression. PL was also found to augment doxorubicin (Dox)-induced apoptosis in prostate cancer (LNCaP cells) [30]. PL and Dox do not induce apoptosis, but a combination of low doses of PL and Dox resulted in a synergistic effect on apoptosis induction. In this process, caspases 8, 3, BID were activated. JNK is also activated in response to PL or combination treatment. The antitumor effect derived from apoptosis induction was also demonstrated in mouse and human lung cancer cells [31]. In response to a high-dose PL treatment, lung cancer cells underwent apoptosis accompanied by the activation of caspase 3 and cytochrome C release. The addition of a caspase inhibitor (Z-VADfmk) completely suppressed apoptotic processes meditated by high-dose PL in lung cancer cells. An in vivo study showed the antitumor property of PL is to induce apoptosis in athymic nude mice model inoculated with prostate cancer DU145 or PC3 cells [32]. Injection of PL caused dramatic tumor regression by triggering apoptotic processes, as demonstrated by histopathology, immunohistochemistry (TUNEL and caspase3), and Annexin V assays. These studies indicated that the upregulation of apoptosis or resensitizing cells to apoptotic stimulation is a promising potential cancer therapy.

Inhibition of Angiogenesis

Angiogenesis refers to the formation of new blood capillaries from existing ones. This complex process involves degradation of the extracellular matrix, migration and proliferation of endothelial cells, tube formation and sprouting of new capillary branches [33]. Angiogenesis is necessary for both cancer growth and tumor transplantation and metastasis. Therefore, inhibition of angiogenesis can be a potential strategy to suppress tumor growth and metastases.

The anti-angiogenic activity of PL was measured using a Chorioallantoic Membrane (CAM) assay, which is used for detecting in vivo angiogenesis. PL showed potent, dose-dependent anti-angiogenic activity in the CAM assay [34]. However, the mechanism by which PL induces anti-angiogenesis was not fully addressed. A recent study [35] found that PLME (the methanol extract of PL) had anti-angiogenic effects through inhibition of human umbilical vein endothelial cell proliferation, migration, and assembly into capillary-like structures. These effects were mediated by the inhibition of VEGFR-2 phosphorylation. In addition, when human aortic endothelial cells were treated with PL or with conditioned media from the breast cancer cell line MDA-MB-231treated with PL (0–0.5 mg/ml), PL significantly suppressed capillary morphogenesis of human aortic endothelial cells in a dose-dependent manner, compared with conditioned media from cells not treated with PL. This anti-angiogenic effect of PL was reported to be due to suppression of AKT activity, which inhibits secretion of VEGF from breast cancer cells [36]. These results demonstrate that PL might be a novel inhibitor of angiogenesis, suggesting that PL could be a new alternative therapeutic agent for angiogenesis-mediated tumor treatment.

Synergetic Effects with Chemotherapeutic Agents

A potentially applicable clinical strategy for PL might be using PL as an adjuvant in combination with cytotoxic anticancer agents. However, clinical studies on the feasibility and rationale of PL as a combination anti-cancer treatment are lacking, with only a single in vitro study available. Collins et al. [30] explored the potential combination treatment of Dox and PL against prostate cancer based on studies suggesting that Dox can synergize with TRAIL to elicit cytotoxicity in various tumors [37-40]. PL also inhibited tumor growth. This study showed that PL might act by interfering with the antiapoptotic factor c-FLIP to enhance sensitization of the Dox-mediated apoptotic signal pathway. The results suggest that a combination of PL with a cytotoxic agent could have synergistic anticancer effects in patients. Similarly, Guo et al. [31] demonstrated a synergistic effect of PL in combination with Dox in lung cancer cells. Cells were treated with PL (0.5 mg/mL), a low dose of Dox (0.5 mg/mL) alone, or a combination of PL and Dox. Treatment with PL or Dox, or the combination of PL and Dox failed to induce apoptosis in normal lung epithelial cells. Lung cancer cells (LKR or H5800 cells) were also insensitive to either single treatment. However, the combination of the two reagents increased the percentage of DNA fragmentation in cancer cells (more than20%). This indicated the occurrence of apoptosis, suggesting that PL and Dox have a synergistic effect on the induction of apoptosis in lung cancer cells. Another potential use of the genus *Phellinus* as an adjuvant combination therapy with conventional anticancer treatments was demonstrated by *Phellinus igniarius*, in the same family as *P. linteus*. *P. igniarius* ethanol extract at 25 or 50 ug/mL in combination with

oxaliplatin or 5-fluorouracil synergistically inhibited the proliferation of human hepatocellular carcinoma cells (SK-Hep-1) [41].

Antitumor Activity of Mushroom Derivatives on Pancreatic Cancer

Till now, no study has been performed about the anticancer effect of PL, however, there are a few experimental data supporting potential anti-tumor activity of mushroom against pancreatic cancer. Simply, although it was not related to PL, another mushroom extract from *Cyathusstriatus* was shown to inhibit the viability of human pancreatic adenocarcinoma cells; HPAF-II and PL 45. It was noted that growth inhibition could be achieved even in low concentration with short exposure period [42]. The growth of pancreatic cancer BXPC3 cells also were found to markedly be inhibited by MMH01, a compound isolated from *Antrodiacinnamomea*. In BxPC3 cells, MMH01 treatment resulted in ballooning, a unique morphologic changes known as an early apoptosis. This morphologic changes, but no DNA fragmentation suggested the mode of cell death induced by MMH01 in pancreatic cacnerBxPC3 might be apoptosis-associated events of necrosis [43].

Recently, the underlying potential mechanisms were actively investigated. Yu et al. [44] demonstrated that antroquinonol, a ubiquinone derivative isolated from a camphor tree mushroom, *Antrodiacamphorata*, induced a concentration-dependent inhibition of cell proliferation in pancreatic cancer PANC-1 and AsPC-1 cells. This anticancer activity in human pancreatic cancers was found to be through an inhibitory effect on PI3-kinase/Akt/mTOR pathways, which results in down-regulation of cell cycle regulators. It was also noted that antroquinonol induces the cross talk between apoptosis, autophagic cell death and accelerated senescence by up-regulation of $p21^{WAF/Cip1}$ and K-ras. With regarding cyclin-dependent kinase inhibitor, $p21^{WAF/Cip1}$, Rosendahl et al. [45] also found that Polysaccharide K (PSK) increases $p21^{WAF/Cip1}$ and promotes apoptosis in pancreatic cancer cells. They showed the antiproliferative action of PSK in BxPC-3, PANC-1, MIAPaCa-2, and AsPC-1 was associated with up-regulated cell cycle regulatory $p21^{WAF/Cip1}$ and pro-apoptotic protein Bax levels, resulting in cell cycle arrest and induction of apoptosis.

Another study evaluated the antitumor mechanism of protein pound polysaccharide under hypoxia because pancreatic cancer is known to be related with high level of hypoxia. Based on the previous observation that HIF-1α and Hedgehog (Hh) signal pathway independently regulate the proliferation and invasiveness under the hypoxia [46], Onish et al [47], demonstrated PSK decreased proliferation in PDAC cells under hypoxia, and it definitely inhibited invasiveness of human pancreatic ductal cell lines (ASPC-1, and SUIT-2). PSK was also noted to decrease the expression of HIF-1α and hedgehog (Hh) signaling-related molecules under hypoxia, in turn, this inhibition of HIF-1α and Hh signaling reduced proliferation and invasiveness in PDAC cells under hypoxic condition.

PSK from *Basidiomycete Coriolusversicolr* also shown to inhibit pancreatic cell line (NOR-P1) by down-regulation of TGF-β1 and MMPs. TGF-β1, the predominant from in human and the most widely studied isoform of TGF-βs, is potent invasion-promoting factors. MMPs are implicated in tumor cell invasiveness. Zhang et al. [48] observed that PSK significantly decreased the invasiveness of NOR-P1 cancer cells in Martrigel-coated filters, and decreased invasion was associated with inhibition of TGF- β1, and MMPs.

PSK-induced indirect antitumor activity by augmenting immune system was investigated in early 1990. Noguchi et al. [49] found that PSK directly augments the proliferation and cytotoxicity of tumor-infiltrating lymphocytes (TILs). TILs defined as lymphocytes found within the tumor tissue and are known to develop as manifestations of the recognition and defense against malignant cells by the host immune system. Therefore, the potential role of TILs has been demonstrated by the prognosis of TILs in pancreatic cancers [50-52]. They observed that DNA synthesis of TILs was increased by incubation with PSK, which was similar to serum level with oral administration of PSK in cancer patients. Although PSK did not affect the cytotoxic activity of TILs against autologous tumor cells in the 4h ^{51}Cr release assay, PSK induced high lysability in the 16-h ^{51}Cr release assay. The DNA synthesis of tumor cells was more suppressed by the mixed-tumor cell culture supernatants of TILs cultured with PSK, compared to that of TILs cultured without PSK, suggesting that PSK induced killing activity of TILs by induction of cytotoxic cytokines.

In early 2000, Zhang et al. [53] also demonstrated PSK-mediated NF-κB inhibition enhanced docetaxel-induced apoptosis in human pancreatic cancer cells. Although PSK alone did not show an effect on the viability of human pancreatic NOR-P1 cancer cells, it dose-dependently enhanced docetaxel-induced apoptosis, suggesting combination of PSK with a low dose of docetaxel may be a new therapeutic strategy to treat pancreatic cancer patients. However, there is no clinical study evaluating the potential role of mushroom derivatives in clinical practice of treating pancreatic cancer. In 1987, only two cases of unresectable pancreatic cancer responding to combined chemotherapy with cisplatin, PSK, and UFT were reported [54].

Oncologic Impact of Polysaccharides from *Phellinus linteus* (Aclang®) in with Postoperative Adjuvant Chemotherapy in Pancreatic Cancer after Surgical Resection: A retrospective pilot study

In Korea, commercialized polysaccharides from *P. linteus* (Aclang, Kwang Dong Pharmaceutical Co., Ltd, Korea) are available for clinical use and are covered by medical insurance during postoperative chemotherapy for gastrointestinal malignancy. In this study, we retrospectively investigated the potential oncologic impact of Aclang with postoperative adjuvant chemotherapy in pancreatic cancer with surgical resection.

A total of 103 patients underwent surgical resection for ductal adenocarcinoma of the pancreas at Severance Hospital, Seoul, Korea, from January 2006 through December 2010. We retrospectively analyzed the medical records of the 53 patients who received postoperative adjuvant chemotherapy without neoadjuvant chemotherapy. In a subset of these patients, Aclang was given concomitantly with postoperative chemotherapy. Two chemotherapy regimens were used: gemcitabine-based therapy and 5-FU-based therapy. Gemcitabine was administered at 1000 mg/m2 as a 60-minute intravenous infusion on days 1, 8, and 15 over a four-week cycle. Cisplatin (70 mg/m2) was sometimes added to the gemcitabine infusion on day 1. For 5-FU based chemotherapy, 5-FU was administered intravenously at 1000 mg/m2 on days 1, 2, and 3 of the cycle, and carboplatin (350 mg/m2) was added on day 2 of the cycle. Aclang (1100 mg, 550 mg/capsule x 2) was administered orally 3 times per day for 2–4 months during postoperative chemotherapy. We divided patients into two groups according to Aclang use. The clinicopathological features of the two groups were compared using Mann-Whitney U-tests for continuous data, and are presented as median values (ranges). Chi-squared tests or Fisher's exact tests for categorical variables were used as appropriate. Disease-free and overall

survival curves were obtained using the Kaplan-Meier method, and differences in survival between groups were compared using a log-rank test. Statistical analysis was conducted using SPSS software version 18 for Windows (SPSS Inc. Chicago, IL, USA). P <0.05 was considered to be statistically significant.

Clinicopathological analysis of postoperative polysaccharide *Phellinus linteus* (Aclang)

From January 2006 through December 2010, 103 patients underwent pancreatectomy for pancreatic ductal adenocarcinoma. A total of 53 underwent postoperative adjuvant chemotherapy without neoadjuvant chemoradiation therapy and were included in this study. Most patients (39, 73.6%) received gemcitabine-based postoperative adjuvant chemotherapy, and the remainder received 5FU-based postoperative adjuvant chemotherapy. Aclang was given to 35 patients during postoperative chemotherapy treatment. Median disease-free survival was 6.9 months (95% Confidence Interval [CI]; 4.6-9.2), and median overall disease-specific survival was 42.3 months (95% CI; 22.8-61.8).Our comparative analysis showed no clinicopathological differences between groups (Table 2). Perioperative and pathological characteristics including T-stage, N-stage, lymphovascular invasion, perineural invasion, tumor grade, and margin status were similar between the groups.

Oncologic impact of Aclang with postoperative chemotherapy in pancreatic cancer after surgical resection.

The anticancer effects of polysaccharides from Aclang were estimated using survival outcomes for each group. Clinicopathological differences were not seen between groups. However, disease-free survival was significantly different for postoperative use of Aclang with adjuvant chemotherapy. While median disease-free survival was 2.8 months (95% confidence interval; 0.143-5.457) in patients that did not receive Aclang®, median disease-free survival was 11.0 months (95% CI; 5.329-16.738, P = 0.017) in patients treated with Aclang. However, overall disease-specific survival was not significantly different between groups (Figure 2).

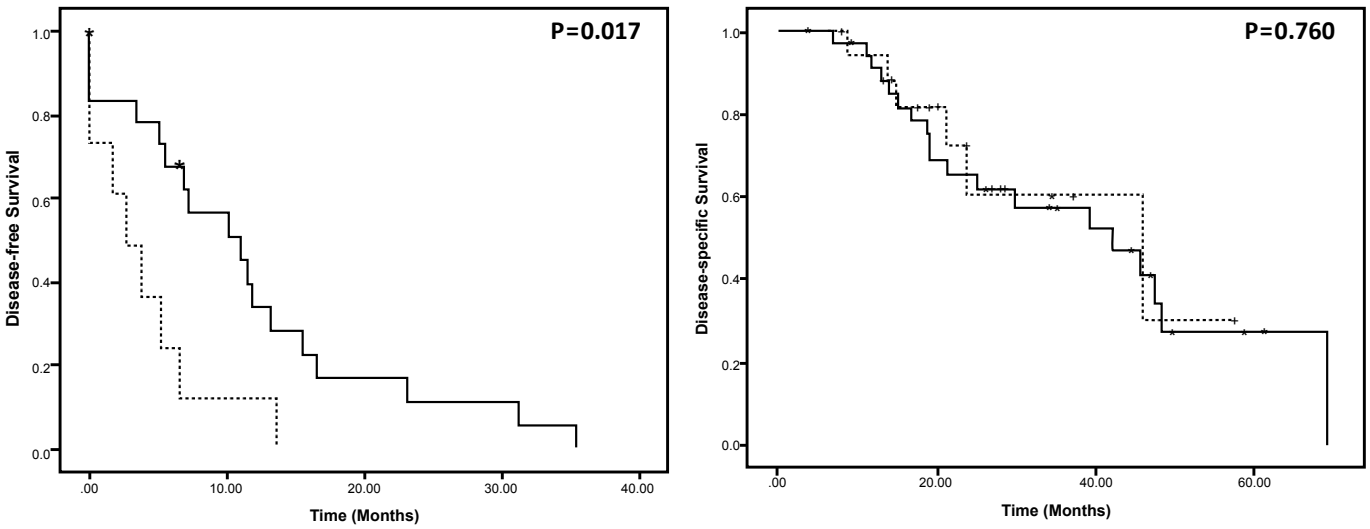
Summary comments

To the best of our knowledge, this study is the first clinical report suggesting that PL could potentially be used to treat pancreatic cancer. Despite that postoperative use of Aclang with postoperative adjuvant chemotherapy was observed to significantly delay tumor recurrence, overall disease-specific survival was similar. Moreover, median disease-free survival (DFS) (2.8 months in control group vs. 11.0 months in à Aclang group) appears to be too short and does not appear to be beneficial oncologic impact when comparing to DFS of the 6.9 months in the surgery-alone group vs.13.4 months in patients with surgery followed by the administration of gemcitabine on CONKO-001 trial [55]. Especially, in treating patients with recurrence, most of patients received different chemotherapeutic agents including DDP, oxaliplatinc, TS-1, Xeloda, and combined radiotherapy. In some cases of local liver metastasis, radiofrequency ablations or transarterial chemoembolization with systemic chemotherapy were performed. Various chemotherapeutic agents were heterogeneously applied according to patients' general condition and preference of the oncologist. Therefore, it cannot be emphasized that PL has definite oncologic role in treating pancreatic cancer because this study is

		Control group (n = 18)	Aclang group (n = 35)	*P-value*
Age (years)		63.2 ± 9.5	62.5 ± 9.0	0.749
Sex	Female/Male	8/10	14/21	0.756
Biliary decompression	No/Yes	12/6	20/15	0.502
Tumor Location	Head/ Body+Tail	11/7	23/12	0.590
Resectability[1]	PR/BR/LA	14/4/0	23/11/1	0.602
Operation	PD/ DPS/TP	10(8)*/7/1	23(21)*/12/0	0.392
Vascular resection[2]	No/Yes	16/1	29/6	0.576
Blood loss (ml)		709.4 ± 556.9	660.2 ± 423.1	0.667
Transfusion	No/ Yes	12/5	25/10	0.726
Operation time (min)	No/ Yes	423.3 ± 192.2	426.0 ± 171.9	0.843
Tumor Size (cm)		2.7 ± 1.8	2.9 ± 1.3	0.585
T-stage	T1/T2/T3/T4	0/0/17/0	1/1/31/2	0.510
N-stage	N0/N1	7/10	13/22	0.964
PNI	No/ Yes	6/11	11/23	0.781
LVI	No/ Yes	12/5	19/15	0.655
Tumor Grade	Well	6	5	0.466
	Moderate	11	26	
	Poor	1	3	
	Undifferentiated	0	1	
R0 resection	R0/R1/R2	15/2/0	29/3/3	0.590
Postoperative chemotherapy	GEM-based/ 5FU-based	11/7	28/7	0.198
LOH (days)		22.6 ± 12.1	19.9 ± 9.7	0.553
POPF	No/ Yes	15/2	34/1	0.915
Mortality	No/ Yes	17/0	35/0	NA

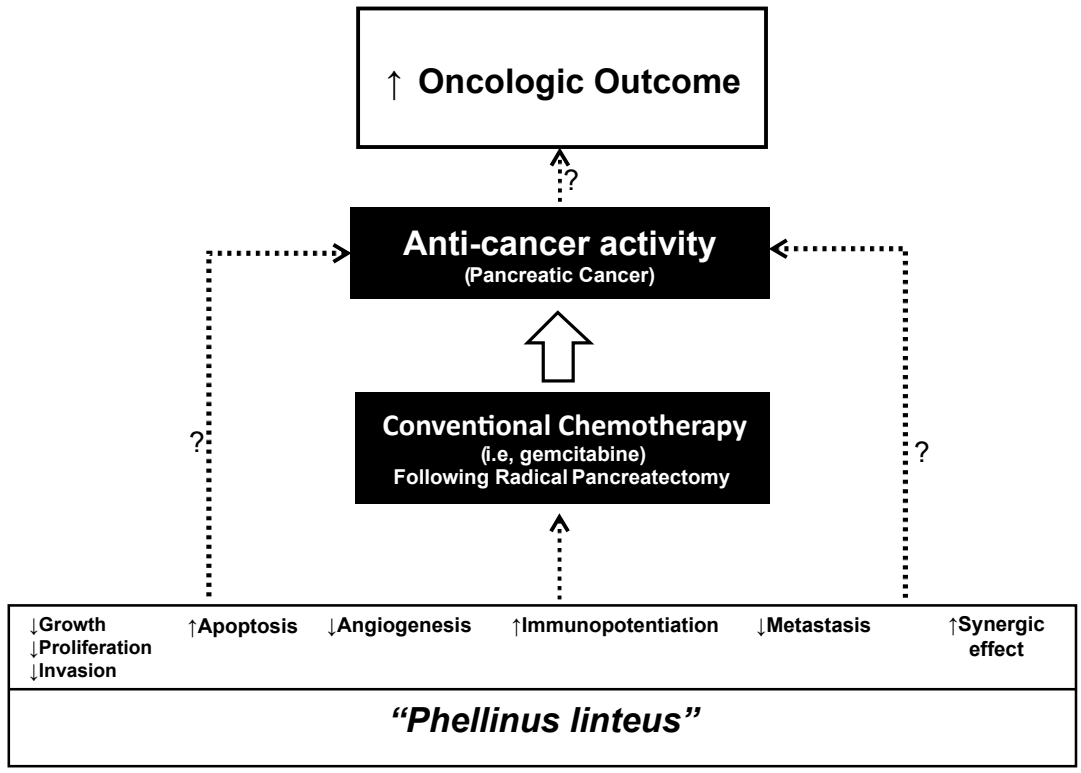
[1]2010 NCCN guidelines, PR=potentially resectable, BR=borderline resectable, LA=locally advanced
[2]Portal vein wedge resection (1) in the control group vs. portal vein segmental resection, (2) wedge resection, or (3) in the Aclang group. * ; indicates pylorus-preserving procedure. PNI; perineural invasion, LVI; lymphovascular invasion, LOH; length of hospital stay, POPF; postoperative pancreatic fistula

Table 2: Clinicopathological features of patients in Aclang and control groups.



Solid line (Aclang group), dotted line (Control group)

Figure 2: Oncologic outcomes according to use of polysaccharide *Phellinus linteus* (Aclang®)



The anticancer effect of *Phellinus linteus* (PL) has been studied, but use of PL for pancreatic cancer seems to be an unexplored area. The pilot study presented here encurages further investigation on PL for pancreatic cancer treatment.

Figure 3: Unveiled potential oncologic role of *Phellinus linteus* in pancreatic cancer treatment.

a retrospective and pilot study based on small patients' number. However, as long as this pilot study showed the statistical difference in oncologic outcome was observed, between two groups, and there are several published scientific back ground for PL and some mushroom derivatives from mushroom in treating pancreatic cancer, we cannot overlook this observation. It should be re-evaluated in prospective randomized control study in near future. We cannot be sure that Aclang had anticancer effects in pancreatic cancer. However, our pilot study provides clinical clues for performing future randomized control studies on the potential of PL for treating resected pancreatic cancer.

Conclusions

Given the poor survival rates of patients with pancreatic cancer, a critical need exists to develop alternative treatments to improve oncologic outcomes of pancreatic cancer. In spite of accumulating evidence for the antitumor effect of PL and mushroom derivatives, until now, no studies have examined the potential anticancer mechanism of PL in pancreatic cancer. Clinical research on the potential of PL for pancreatic cancer treatment is sparse. In addition, no sound evidence does exists on the proper dosage and treatment period for PL as an adjuvant agent in managing pancreatic cancer. Therefore, future studies need to validate the oncologic impact of PL on resected pancreatic cancer. In addition, the exact mechanisms of action of PL in pancreatic cancer pathogenesis should be investigated (Figure 3).

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