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Effect of Telomerase Peptide Vaccination, GV 1001 Combined with Gemcitabine in Treatment of Pancreatic Ductal Adenocarcinoma

췌장암에서 GV 1001와 Gemcitabine 복합요법에 의한 효과

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by

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ABSTRACT

Introduction: Pancreatic ductal adenocarcinoma (PDAC) shows dismal prognosis due to early metastasis, frequent recurrence and chemo-resistance. However, there is no effective treatment to overcome these problems. GV1001 is a telomerase-based cancer vaccine made of a 16-mer TERT peptide and human telomerase reverse transcriptase (hTERT), the rate-limiting subunit of the telomerase complex, is therefore an attractive target for cancer vaccination. The aim of this study was to evaluate the combination benefit of telomerase peptide vaccination, GV1001 combined with gemcitabine in PDAC.

Methods: Human pancreatic cancer cell lines (Panc-1 and AsPC-1) were used in vitro experiment and also, PDAC xenograft mice model was established using pancreatic cancer cell lines (Panc-1 and AsPC-1). Treatment groups were divided as follows; control, gemcitabine alone, GV 1001 alone, gemcitabine and GV 1001 combination. The changes of weight and tumor size were evaluated in regular intervals before and after the treatment. The inflammatory cytokines (IL-1β, IL-6, TNF-α, INF-γ), leptin and ghrelin were measured from the serum of xenograft tumor mice model.

Result

In vitro experiments: GV1001 treatment alone did not affect the proliferation or the apoptosis of PDAC cells.

In vivo experiments: Mean tumor volume and size were decreased in treatment of gemcitabine alone group and gemcitabine with GV 1001 group, and there were no significant differences between the two groups. However, gemcitabine alone or gemcitabine with GV 1001 treatment groups had significantly small tumor size and volume compared to control group ($P < 0.001$). Interestingly, there was a significant difference in mean body weight between the groups with gemcitabine alone vs. gemcitabine with GV 1001 combination groups. Mice of gemcitabine with GV 1001 treatment group did not have significant weight loss compared to gemcitabine alone group although they have decreased tumor size and volume. Serum level of IL-1β showed significant decrease in groups with GV1001 combination with gemcitabine and GV1001 only ($p=0.01$). On the other hands, serum level of IFN –γ and TNF-α significantly increased in groups with GV1001 including treatment ($p<0.001$). Treatment groups with gemcitabine alone and gemcitabine combined with GV1001 had significant amount of reduced tumor size and abundant apoptosis from the evaluation of xenograft tumor specimens after the sacrifice. Surprisingly, xenograft tumor tissue in gemcitabine single treatment group showed tumor tissue had been replaced by severe fibrosis whereas gemcitabine combined with GV1001 treatment group had significant reduced fibrosis.

Conclusions: GV1001 showed beneficial effects combined with gemcitabine in PDAC xenograft mice model preventing from emaciation. Moreover, GV 1001 combined with gemcitabine treatment showed significant loss of fibrosis

in tumor tissue as well as tumor cell death. Therefore, further investigation of GV1001 effect may give us useful insights to understand the biology of PDAC progression and the synergistic effects of anti-cancer drug delivery in PDAC treatment.

Keywords: GV 1001, gemcitabine, xenograft tumor model, pancreatic ductal adenocarcinoma

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(B) Xenograft PDAC tumor established by PANC1

(Masson's trichrome staining X400)

LIST OF ABBREVIATIONS

PDAC: pancreatic ductal adenocarcinoma

hTERT: human telomerase reverse transcriptase

DMEM: Dulbecco modified Eagle medium

FBS: fetal bovine serum

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is a very aggressive human cancer and has dismal prognosis with only 6% of patients survive 5 years after diagnosis.[1-4] In spite of the progresses of treatments, the attempts at improving survival of patients with PDAC in the past 15 years, especially in the advanced disease setting, have failed and resulted in no significant improvement.[5] Surgical resection is the only potentially curative treatment and only 15% of patients could be candidate for resection.[6,7] Some chemotherapeutic agents have been used in treatment of PDACs, and gemcitabine became the standard chemotherapeutic agent in pancreatic cancer after randomized trial in 1997.[8] Gemcitabine is a nucleoside pyrimidine analogue which exerts its cytotoxic actions primarily by the incorporation of gemcitabine triphosphate into DNA, leading to masked chain termination.[9] However, pancreatic cancer is highly resistant to chemotherapy including gemcitabine, and the most disappointing circumstance is mainly due to the late diagnosis of PDAC.[10,11] In addition, the best supportive care and maintain the better quality of life are critical since the majority of the patients with PDACs are in advanced stage. To date, many efforts to find an appropriate combination of multi-therapeutic agents with different modes of action to overcome the chemoresistance have been made during the past years. The most of these chemotherapeutic agents have not been successful enough to show the significant survival benefit.[12-18] Thus, more effective treatment strategies are highly required, and immunotherapy for target which is critical in cancer growth seemed to be a promising approach.[19,20]

In the process of repeated rounds of DNA replication, the telomeric ends of DNA become progressively shortened and without a compensatory mechanism cells senesce and die.[21,22] Therefore, telomerase expression is essential for the proliferation of most cancer cells, but the enzyme is inactive in the majority of normal human tissues. Reactivation of telomerase, the telomere-repair enzyme, is a crucial event in oncogenic transformation, and is highly expressed in essentially all cancer forms, while the expression in normal tissues is restricted. [23] Moreover, telomerase activity is considered indispensable for tumor immortalization and growth and occurs in nearly all pancreatic cancers. [23] That means that inhibiting the chromosome-elongating enzyme should, in theory, be a relatively safe and effective way to, if not directly kill, at least weaken cancer cells before treating with other agents. Therefore, human telomerase reverse transcriptase (hTERT), the rate-limiting subunit of the telomerase complex, is an attractive target for cancer vaccination.

GV1001 is a human telomerase reverse transcriptase catalytic subunit (hTERT) class II 16mer peptide vaccine.[21,22] Bernhardt et al reported in their phase 2 trial that GV1001 treatment in advanced pancreatic cancer showed a total immune response in 24 (63%) of 38 patients and those responders had a greater median survival (216 days) than did non-responders (88 days).[24] Although, cytotoxic drugs are generally regarded as immunosuppressive, some chemotherapy regimens might potentiate the effect of cancer vaccines.[25-30] The preclinical data clearly showed immunogenicity of GV1001

in patients with PDACs that the synergy of gemcitabine with cancer vaccines and the other positive immunomodulatory effects of gemcitabine and fluoropyrimidines.[23] Therefore, the aims of this study were to investigate the effect of telomerase peptide vaccination, GV 1001 combined with gemcitabine in treatment of PDAC.

Materials and methods

Pancreatic cancer cell lines

Human pancreatic cancer cell lines, Panc-1 and AsPC-1 were obtained from Korea Cell Line Bank and maintained in RPMI1640 medium (WELGENE, Daegu, Korea) containing 10% heat-inactivated fetal bovine serum (Life Technologies, Grand Island, NY, USA) and antibiotics (100 U/ml of penicillin and 100 μg/ml streptomycin; Life Technologies). They were incubated at 37°C and 5% CO2.

Apoptosis determination

Panc-1 and AsPC-1 were cultured and divided into 3 groups according to the treatment: (i) control, (ii) GV1001 (20 μM) alone, (iii) GV1001 (40 μM) alone. PDAC cell lines were cultured for 24 hrs and washed wit cold PBS and then resuspended in 1× Annexin V binding buffer (BD Biosciences, San Jose, CA, USA) at a concentration of 1×10^6/ml. After PDAC cells were incubated with annexin V-fluorescein isothiocyanate for 15 minutes at room temperature in the dark and 7AAD (BD Biosciences), degree of apoptosis was analyzed by fluorescence activated cell sorting (FACS). The proportion of stained cells in each quadrant was quantified with CellQuest software (BD Biosciences).

Cell proliferation assay

Cells were plated into 96-well plates at a density of 5 x 10^3 cells/well and cultured in the presence or absence of GV1001 in various concentration from 0.005 nM to 5,000nM for 72 hrs. Cell proliferation was then measured with the Cell Counting Kit-8 (CCK-8) assay (Dojindo, Kumamoto, Japan) according to the manufacturer's instruction. Absorbance values were measured using the microplate reader and SoftmaxPro software (Molecular Devices, Sunnyvale, CA, USA).

Animals

Seven-week-old male BALB/c nude mice were purchased from Orient (Gyeonggi-do, Korea). Mice were housed under specific pathogen-free conditions, and a γ-ray-irradiated laboratory rodent diet (Purina Korea, Gyeonggi-do, Korea) and autoclaved water were provided ad libitum. All the protocols for the animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee at Seoul National University Hospital (IACUC No. 13-0717). All animal procedures were in consistent with the "Guide for the Care and Use of Laboratory Animals" issued by the Institute of Laboratory Animal Resources Commission on Life Science, US National Research Council.

Establishment of PDAC xenograft tumor model and treatment

To generate tumors, human PDAC cells (Panc-1 and AsPC1, 1 x 10^6 cells/50 μl PBS) were subcutaneously inoculated with 50 μl of Matrigel (Figure 1) in both sides of buttocks. All mice were divided into 4 groups randomly with 5 mice in each group. The treatment was began after 10 days from the PDAC

cell injection with confirmation of gross xenograft tumor in each mouse (Figure 2): (i) control (vehicle alone), (ii) gemcitabine (twice-a-week intraperitoneal injection at 125 mg/kg for 2 weeks), (iii) GV1001 (every day subcutaneous injection at 50 μg/ea for 2 weeks), (iv) gemcitabine (twice-a-week intraperitoneal injection of at 125 mg/kg for 2 weeks) and GV1001 (every day subcutaneous injection at 50 μg /ea for 2 weeks).

Monitoring and measurement of tumor growth

The body weight and the tumor size of each mouse were measured every other day using electronic scale and caliper. Tumor volume was calculated by the following formula: tumor volume = (length x width^2) x $\pi/6$.[31]

Blood sample and analysis of serums

Blood samples were collected from the infra-orbital venous plexus at the time of sacrifice. Four cytokines were analyzed for each group of mice. The cytokines analyzed were interleukin (IL)-1β, IL-6, interferon (IFN)- γ and tumor necrosis factor (TNF)- α. Quantikine human enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D systems (Minneapolis, MN, USA). Analyses were performed according to the manufacture's protocol for each ELISA kit, assayed in triplicate, and read on a Molecular Devices microplate reader at 450 nm (Menlo Park, CA, USA). To keep experimental values within the linear region of the standard curves, samples were diluted from 1:5 to 1:100 as necessary for stimulated culture samples for IL-1β, IL-6, IFN γ and TNF α. Serum leptin concentration was measured by ELISA, using a commercial kit (mouse leptin (lep) and mouse ghrelin (Ghre) ELISA kit, Cusabio, China) according to manufacturers'

protocol. The detection limit for leptin was 0.08 ng/mL. The intra- and inter-assay coefficients of variation were 8% and 10%, respectively, for the measurement.

Harvest and histololgical examination of the xenograft tumor tissue

The mice were immediately sacrificed after the 2 weeks of treatment protocol (Figure 2). Tumors were excised, weighed, then fixed and processed for paraffin embedding. Tissue sections (5 μm) were prepared using a microtome, placed on glass slides. Serial sections were cut from paraffin-embedded tumor tissue, and stained with H&E and Masson's trichrome. For Masson's trichrome staining, the percentage of fibrotic tissue was expressed as a ratio of the collagen staining area divided by total viable tumor tissue (TVTT) area (excluding necrosis) X 100. These measurements were repeated for a minimum of three sections cut at different levels for each xenograft tumor. Researchers were blinded to sample identity during analysis.

Statistical analysis

All experimental results represent at least 3 independent experiments using cells from a minimum of three separate isolations. Results for continuous variables are expressed as means ± standard error of mean and compared with the Kruskal-Wallis ANOVA followed by Dunn's multiple comparison test or repeated measures ANOVA. $P < 0.05$ was considered statistically significant. Analysis was performed with GraphPad Prism version 5.04 (GraphPad Software Inc., La Jolla, CA).

Results

Direct cytotoxic effect of GV1001 in PDAC cells

Direct cytotoxic effect of GV1001 was evaluated in PDAC cells. FACS analysis was done treated with GV 1001 after 36hrs. In figure 3A & 3B, PDAC cell lines (PANC1, AsPC1) were tested according to the different concentration of GV1001. Therefore, there were 3 groups according to the different concentration of GV1001 treatment (control, 20 uM and 40 uM). Figure 3A showed 5.2%, 3.9% and 4.0% of early apoptotic cells in each group, and figure 3B showed 4.1%, 3.4% and 7%: control, GV1001 20uM and GV1001 40um accordingly. In addition, the number of cells in early stages of apoptosis and the late stage of apoptosis didn't make any significant statistical differences compared to the control group and also according to the concentration of GV1001. In addition, PDAC cells (AsPC1, PANC1) were cultured with GV1001 in various concentrations (Figure 3C). In figure 3C, each PDAC cells (AsPC1, PANC1) were not affected its viabilities via GV100. Therefore, we could observe that GV1001 did not have direct cytotoxic effect to the PDAC cells.

Harvesting xenograft tumors established by PDAC cells

There were 4 different treatment groups and figure 4 showed each different treatment group: control, GV1001, GV1001+ gemcitabine and gemcitabine alone. After the completion of protocol (10 days of tumor growth time + 14 days of treatment period), each group of mouse was sacrificed and examined the gross tumor growth as well as any signs of PDAC cell dissemination. The total of 60 mice (20 mice in pilot study and 40 mice for main study) was used

during this study and the 4 different treatment groups had 5 mice each. They are the followings; 20 mice were used in PDAC xenograft tumor established by AsPC1 and PANC1 PDAC cells respectively. PDAC xenograft tumor was successfully established and all of the study mice had a pair of ovoid PDAC tumors on both sides of buttocks. In addition, there was no treatment related mortalities. However, one mouse was dead before the treatment was begun from gemcitabine + GV 1001 group.

Weight changes among the different treatment groups

The weight of each mouse was measured and the changes of their weight were described in figure 5 & 6. In the xenograft mouse model of AsPC1 and PANC1, the body weight of mice was checked every other day. There was no significant difference in mean body weight between the groups. The changes of PDAC xenograft tumor model established by AsPC1 and PANC1 were described separately in figure 5 and 6. The weight of mice in control and GV1001 treatment only groups didn't change much during the study. The range of body weight in control and GV 1001 were not changed much; control group: median 25.5, range 23-26g, GV1001 group: median 25g, range 23-26g. However, mice in GV1001+ gemcitabine treatment group had sudden decrement of body weight along with the chemotherapeutic agents. Figure 5A and 6A showed that there were significant body weight changes among the different treatment groups and it was statistically significant ($P < 0.0001$). Interestingly, body weight of GV1001 combination with gemcitabine group was not dramatically significant compared to gemcitabine treatment only group.

Tumor volume and tumor volume/body weight changes among the different treatment groups

PDAC xenograft tumors had different tumor size at the end of treatment, and there were clearly demarcated tumors in control and GV1001 only treatment groups. On the other hands, tumors in GV1001+gemcitabine and gemcitabine alone groups were nearly disappeared or significantly shrunk in the gross examination of study mice. The length and width of each tumor were measured and volume was calculated using above mentioned formula: tumor volume = (length x width^2) x $\pi/6$.[31] PDAC xenograft tumors of AsPC1 and PANC1 in the same treatment groups did not show significant difference in terms of tumor volume. However, there was statistically significant volume decrease in gemcitabine alone and gemcitabine+GV1001 treatment groups from the both PDAC xenograft tumors of AsPC1 and PANC1 ($p < 0.0001$, Figure 5C & 6C). In terms of GV1001 effects on PDAC xenograft tumors, GV1001 single treatment did not show anti-cancer effect compared to the control groups (Figure 5C & 6C). Surprisingly, body weight of GV1001+ gemcitabine did not show significant decrease during the treatment compared to gemcitabine treatment only group. Whereas both of treatment groups had significant decrement of tumor size and they didn't show the significant difference in terms of decreased tumor size (Figure 5 & 6).

Serum level of inflammatory cytokines and ghrelin after the treatment of GV1001 combined with gemcitabine

Each treatment group fulfilled the protocol and treated accordingly. At the end of treatment, blood sample of each study mouse was acquired and analyzed

for the following cytokines; IL-1β, IL-6, IFN –γ, TNF-α and leptin and ghrelin as well. Figure 7 showed that IL-1β showed significant decrease in groups with GV1001 combination with gemcitabine and GV1001 only (p=0.01). However, IL-1β was highly produced in gemcitabine alone treatment group and control group. Also, IFN –γ, TNF-α and ghrelin were existed significnatly higher in treatment groups containing GV 1001. However, IL-6 and leptin did not show statistically significant differences among the treatement groups (data not shown).

Pathology of xenograft PDAC tumor after the treatment of GV1001 combined with gemcitabine

After the harvesting PDAC xenograft tumors, H&E staining as well as Masson's trichrome staining were done for each specimen. Gemcitabine alone and gemcitabine+GV1001 groups had significant amount of reduced tumor tissue and apoptosis and it was confirmed in pathologic specimens as well (Figure 8). Although both treatment groups which containing gemcitabine did not show significant difference in tumor size, there was marked difference in terms of fibrosis in pathologic specimens between the two groups. Gemcitabine single treatment group showed the abundant fibrosis replaced the tumor tissue whereas gemcitabine+GV1001 treatment group had significant reduced fibrosis compared to gemcitabine treatment group, however, both groups had significant tumor cell death (Figure 9).

Discussion

GV1001 is a telomerase-based cancer vaccine made of a 16-mer TERT peptide, an attractive target for cancer vaccination and the main purpose of this study was to explore the effect of GV1001 combined with gemcitabine in the treatment of pancreatic ductal adenocarcinoma (PDAC). There were several valuable findings from this study which I would like to address and they are the followings.

First of all, it was observed that GV1001 did not have direct effects on the proliferation or the apoptosis of PDAC cells in vitro experiments and therefore, GV1001 did not have direct anti-cancer effects. GV1001, telomerase peptide vaccine whose mechanism was known to activate combined CD4/CD8 T-cell response and it would depends on antigen-presenting cells (APC).[24] Therefore, it did not show any direct anti-cancer effect in vitro experiment. On the other hands, PDAC xenograft mice model showed that treatment groups with gemcitabine alone and gemcitabine combined with GV1001 had significant tumor reduction compared to other groups. However, it seemed that anti-cancer effect came from gemcitabine since GV1001 alone treatment group did not have significant reduction in tumor size. There was no significant difference in reduced tumor size between the treatment groups with gemcitabine alone vs. gemcitabine+GV1001. Also, there was almost no evidence of gross tumor left in gemcitabine and gemcitabine+GV1001 treatment groups as well. There has been no sufficient amount of studies regarding vaccine therapy showing significant benefit in

advanced pancreatic cancers.[32] Wobser et al. reported that a 77-year-old patient (advanced PDAC with liver metastases) treated with survivin-based peptide vaccination had PR at 6 months and CR at 8 months.[33] However, the patients developed recurrent disease in a short period of time from the cessation of vaccine therapy. However, Phase I/II studies with GV1001 showed the prolonged survival and tolerability.[24] Moreover, not only the TeloVac trial but also a number of different approaches have shown that vaccination to hTERT can elicit immune responses during chemotherapy without clinical efficacy.[34] Also, there have been many studies that some chemotherapies can be combined with immunotherapy in murine models and they improved outcome synergistically.[25,35,36] Middleton et al conducted GV1001 phase III randomized trial and they mentioned some of the possible reasons regarding their failure to show any survival benefit.[34] The lessons from the failure of this phase III clinical trial were as follows; gemcitabine as a single agent in treatment of PDAC had been approved on the basis of clinical benefit response that did not really require significant radiological responses, and the combination with capecitabine added only little to overall radiological response. As a result, there must be not enough cancer cell death to enhance responses to immunotherapy.

Despite of all the disappointing results from other clinical and preclinical trials, this study showed novel effect of GV1001 which was never been reported from the various studies whose aims were to evaluate the effects of GV1001 in PDAC treatment. PDAC xenograft mouse model whose treatment

included GV1001 had excellent performances and did not get emaciated. The objective evidences were the weight changes and intensity of activities during the treatment, and this phenomenon raised new hypothesis that GV1001 might have effects on cancer cachexia and anorexia under the anti-cancer treatment circumstances. Cachexia is known to be one of the most frequent characteristics among the gastrointestinal tract cancers, with up to 50% of treatment naïve patients and approximately one-third of them are losing more than 5% of their original body weight.[37] Also, weight loss is commonly associated and even in early stages of pancreatic cancer patients had relatively significant weight loss than other malignancies[38]. Tissue catabolism in cachexia is well known, and TNF α, IL-1, IL-6 and IFN γ were reported as potential mediators of cachexia.[39-42] Recently, ghrelin has been known for its role as a strong stimulant of food intake and weight gain in humans and it brought attention to be as a treatment agent for the cachexia.[43,44] Therefore, major inflammatory cytokines such as IL-1β, IL-6, IFN γ, TNF α and ghrelin and Leptin were measured from the serum of each mouse after the treatment. Serum level of IL-1β showed significant decrease and IFN –γ and TNF-α were significantly elevated in GV1001 containing treatment groups. Also, there was a tendency of increment in serum level of ghrelin in GV1001 containing treatment groups, however, it was not statistically signifcant among the groups. Whang et al even reported that ghrelin might have a role in promoting PDAC cellular proliferation and invasiveness as well, and this material needs to be further studied.[45]

The most interesting finding in this study was GV1001 effect on stroma of PDAC and its microenvironment. Both treatment groups, gemcitabine alone and gemcitabine combined with GV1001, had significant reduction in tumor size and abundant apoptosis were observed from the xenograft tumor specimens after the sacrifice. Although both treatment groups had significant tumor cell death, tumor specimens of gemcitabine alone treatment had severe fibrosis whereas gemcitabine combined with GV1001 treatment showed significant loss of fibrosis. One of the most difficult obstacles which preventing treatment success of PDACs is an early metastasis with rapidly progressive nature, but other immunological and stromal factors are as important as to be overcome.[46,47] A dense stromal reaction has been shown to impede the penetration of cytotoxics into pancreatic tumors, thus restricting the synergistic potential of chemotherapy.[48] Freig et al reported in a trial of an anti-PD-L1 antibody that objective responses were reported in malignant melanoma, renal-cell cancer, NSCLC and ovarian cancer, but there was no reponsders among the 14 patients with advanced PDACs.[46,49] The relatively poor response in immunotherapy efficacy of PDACs might be related to specific carcinoma-associated fibroblasts (expressing fibroblast activation protein), which secrete CXCL12 and thus stop T cells from accessing cancer cell regions in the stroma.[49,50] In a genetically engineered mouse model of PDAC blocking the receptor of CXCL12, induced rapid T-cell accumulation and synergized with α-PD-L1 in cancer cell killing.[49] Above observations supported that PDAC xenograft tumors responded to GV1001 by reducing tumor fibrosis when they were treated with gemcitabine. Therefore, GV1001

might have robust effects in enhancing drug delivery and become a promising tool to overcome chemoresistance in treatment of PDACs.

This study revealed that GV1001 combined with gemcitabine treatment in PDAC xenograft mice model showed beneficial effects on preventing emaciation as well as maintaining high performance of physical activities, although GV1001 did not have direct anti-cancer effects on PDAC tumors. Furthermore, GV 1001 combined with gemcitabine treatment showed significant loss of fibrosis in tumor tissue as well as tumor cell death, and it might be the key component to understand the possible synergistic effects of anti-cancer drug delivery in PDAC treatment. Here, I would like to address that further investigation of GV1001 effect may give us useful insights of understanding the biology of PDAC progression and the synergistic effects of anti-cancer drug delivery in PDAC treatment.

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Figure 1 Establishment of PDAC xenograft tumor model

PDAC cells (AsPC1, PANC1) were injected to the 4 different groups of BALB/c nude mice in $1X10^6$ each. Treatment arms were waited for 10 days until xenograft tumors were firmly established with a diameter of 5-7mm.

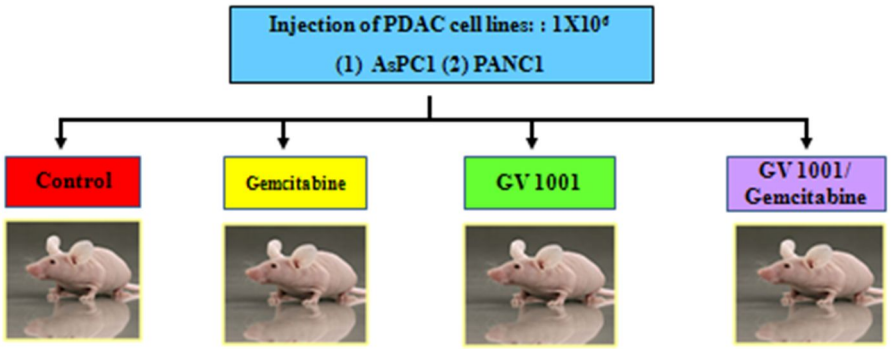


Figure 2 Treatment protocol in PDAC xenograft tumor model

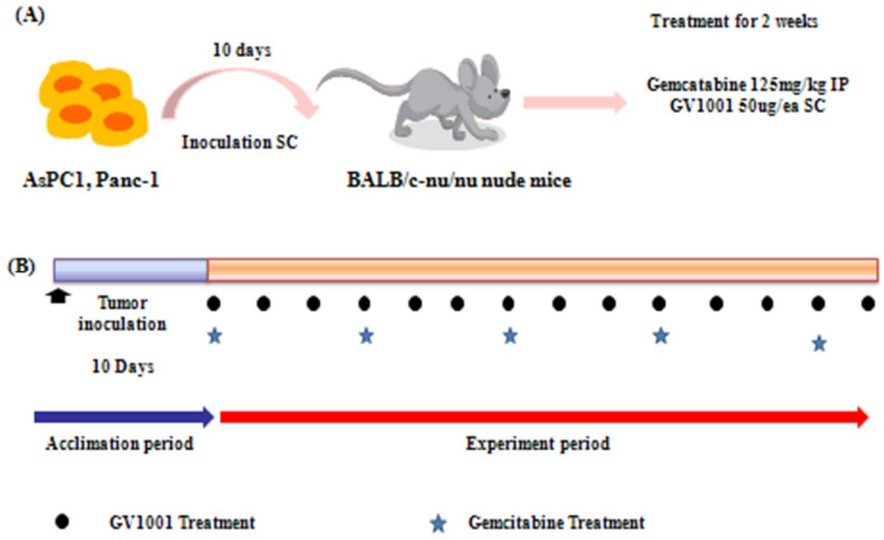
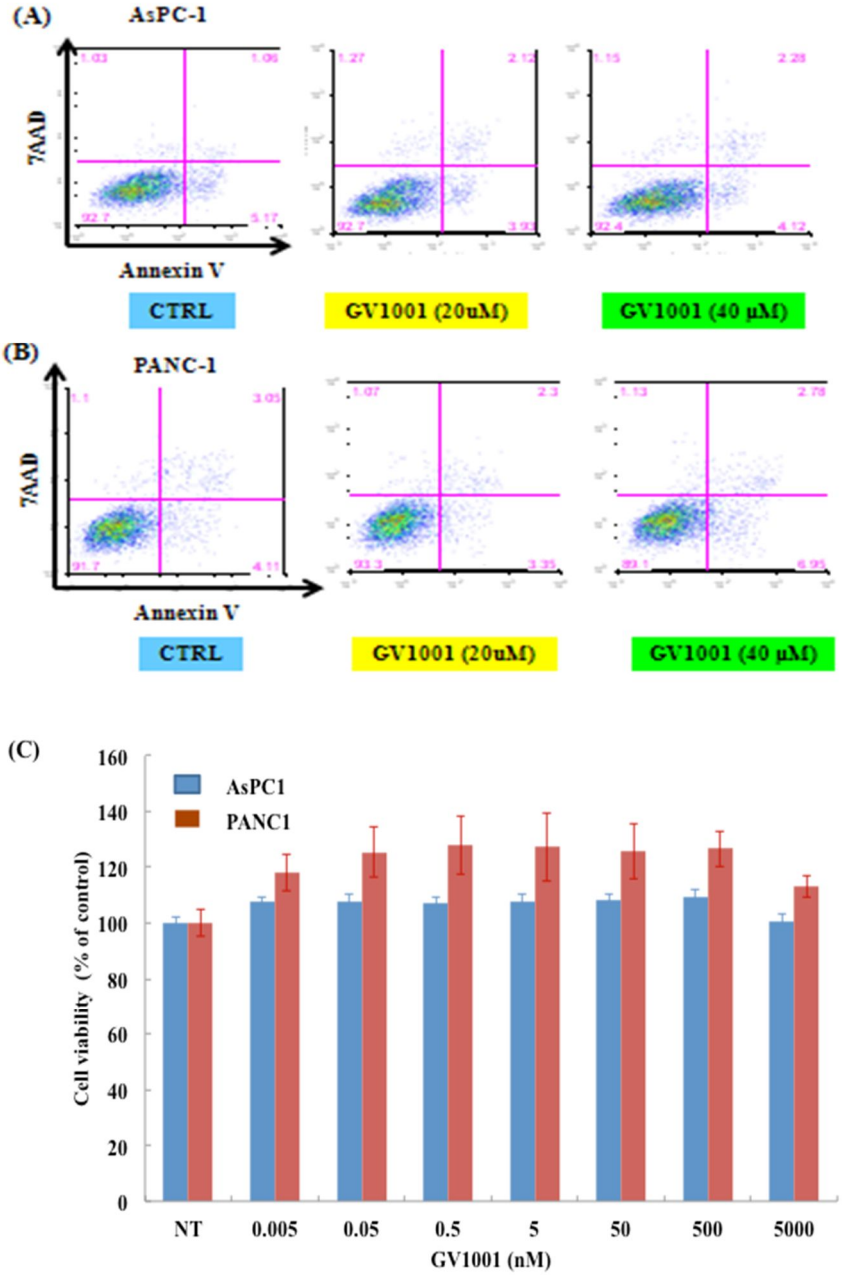


Figure 3 Direct cytotoxic effect of GV1001 in PDAC cells

PDAC cells were co-cultured with different concentration of GV1001 and degree of apoptosis was analyzed by FACS



Figr the different treatment protocol

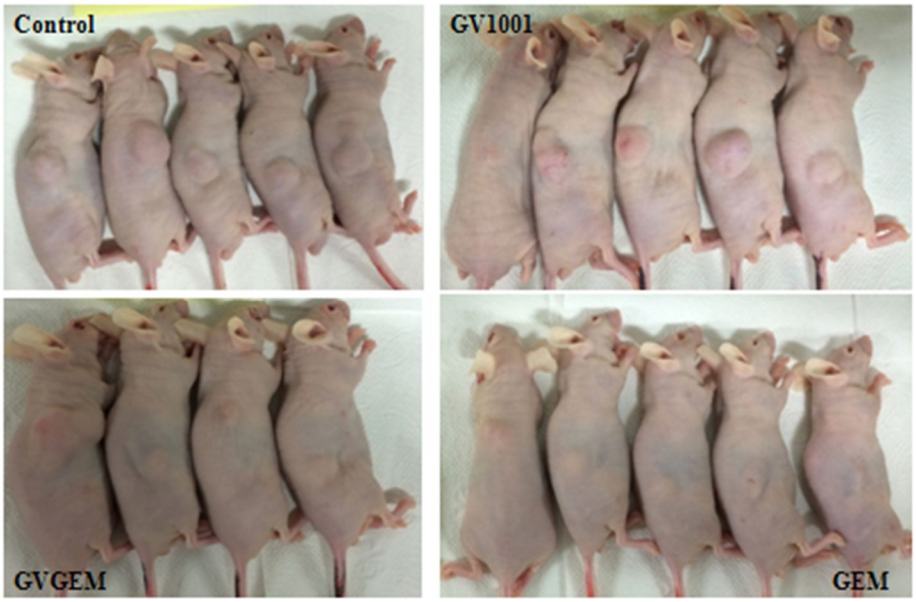


Figure 5 Changes of PDAC xenograft tumor after the treatment: AsPC1

(A) Body weight changes (B) Xenograft tumor after the sacrifice

(C) Tumor volume changes (D) Tumor volume/body weight changes

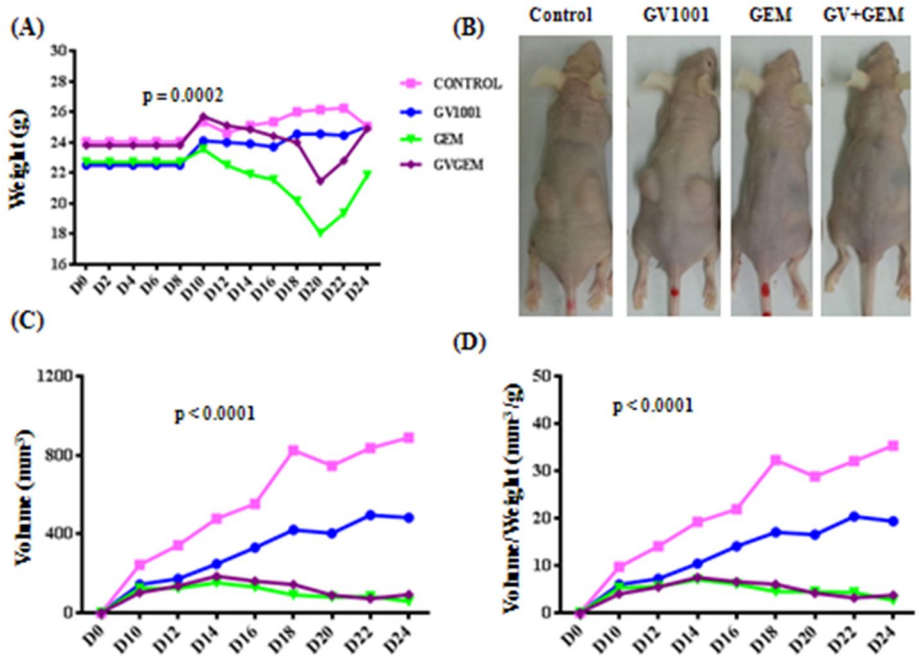


Figure 6 Changes of PDAC xenograft tumor after the treatment: PANC1

(A) Body weight changes (B) Xenograft tumor after the sacrifice

(C) Tumor volume changes (D) Tumor volume/body weight changes

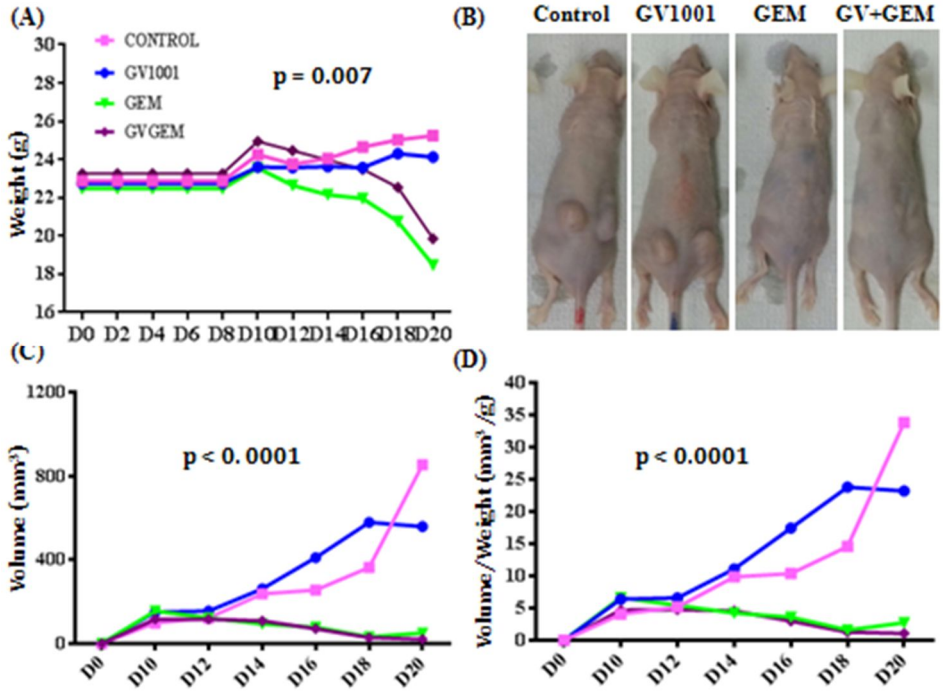


Figure 7 Serum level of inflammatory cytokines and ghrelin after the treatment of GV1001 combined with gemcitabine: in vivo models using AsPC1 and PANC1: (A) IL-1β, (B) IFN –γ, (C) TNF-α, (D) Ghrelin

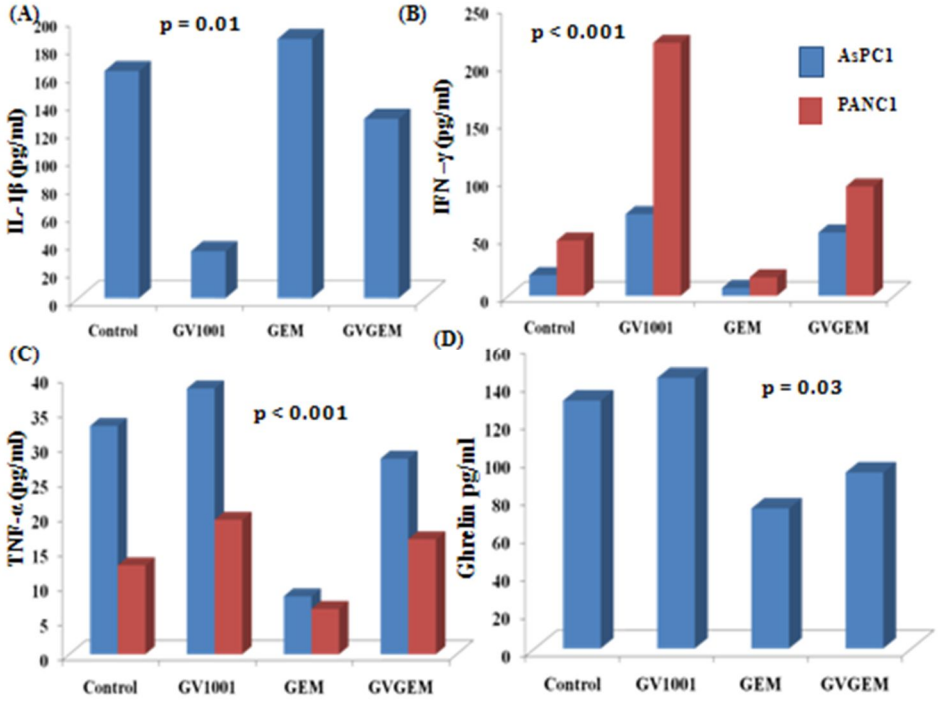


Figure 8 Pathology of xenograft PDAC tumor after the treatment of GV1001 combined with gemcitabine

(A) H&E staining (X100) (B) Masson's Thrichrome stinnig (X100)

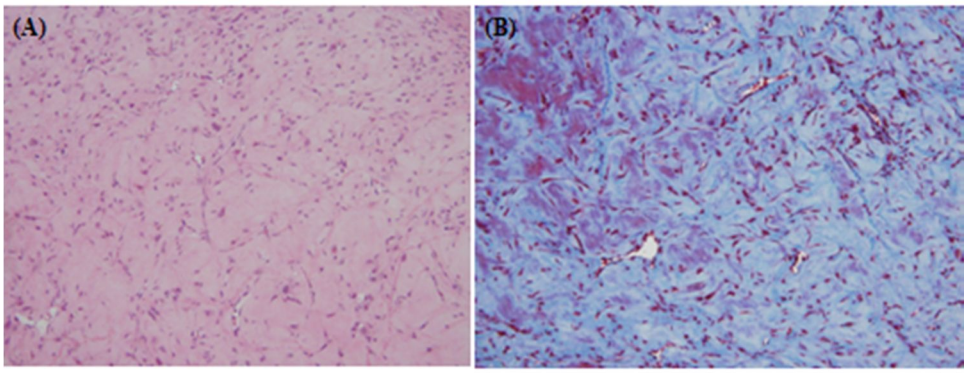
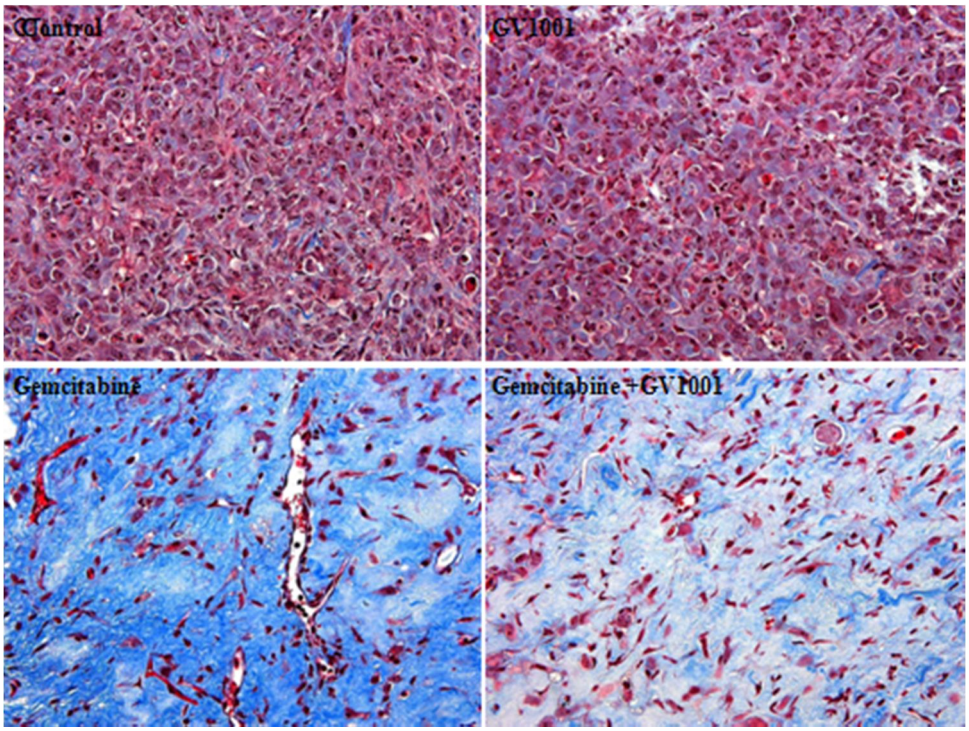


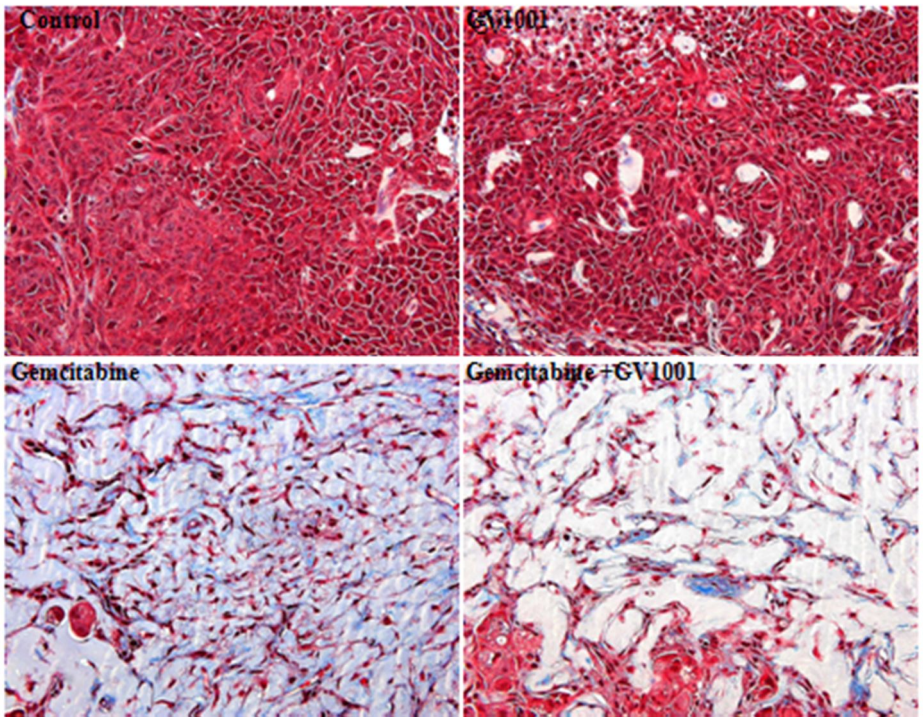
Figure 9 Changes of fibrosis in xenograft PDAC tumor among the different treatment groups: Control vs. GV1001 vs. gemcitabine vs. gemcitabine+GV1001

(A) **Xenograft PDAC tumor established by AsPC1 (Masson's Trichrome staining X400)**



(B) Xenograft PDAC tumor established by PANC1

(Masson's Trichrome staining X400)



국문초록

목적: 대부분의 췌장암 환자가 수술 불가능한 상태에서 발견이 되고 수술 가능하다고 해도 5 년 이내 대부분 재발하게 된다. 빠른 시간 내에 원격 전이 하는 환자에 대해서는 더욱더 효과적인 전신 요법이 매우 시급한 실정이다. 이에 대해 본 연구자들은 GV 1001 (a reverse-transcriptase-subunit of telomerase (hTERT) derived peptide)이라고 알려진 췌장암 vaccine 을 기존 치료제인 gemcitabine 과 병합에서 사용시 이에 대한 항암효과를 평가하고자 하였다.

대상 및 방법: PANC1 및 AsPC1 을 이용하여 GV1001 의 췌장암 세호에 대한 직접적인 사멸 효과를 in vitro model 에서 평가 하였다. 췌장암 세포주를 이용하여 췌장암 이종이식 동물 모델을 확립 후 각각의 약제 단독 요법 및 복합요법의 효과를 in vivo model 에서 평가하였다.

결과: GV 1001 단독 요법으로는 췌장암에 대한 항암 효과는 관찰되지 않았다. 놀랍게도 gemcitabine 및 gemcitabine +GV1001 과 병행한 췌장암 이종이식 동물 모델에서 췌장암에 대한 항암 효과가 두군 모두 효과가 있었으며 gemcitabine 단독으로 투여한 군에서는 누드마우스의 몸무게가 현저히 줄고 활동도가 떨어진 방면 GV1001+ gemcitabine 병합 요법을 시행한 군에서 치료전의 몸무게를 유지하는 것으로 관찰되었다. 이들의 (GV1001+ gemcitabine 병합

요법군) 혈액 샘플에서는 IL-1β 가 의미 있게 감소하였고 TNF-α 및 INF-γ 는 의미 있게 상승하였다. 더불어 ghrelin 이 증가되는 경향을 보였다. 췌장암의 병리학적 소견에서 GV1001+ gemcitabine 병합 투여군이 gemcitabine 단독군에 비해서는 현저히 섬유화가 줄어든 소견이 관찰되었다.

결론: GV1001 이 비록 직접적으로 의미 있는 항암효과를 나타내지는 않았으나 기존의 치료제인 gemcitabine 과 함께 투여할 때 향후 항암제 내성 및 환자의 well being 및 삶의 질 향상에 연관이 있을 가능성이 높아 보이며 상기 신약에 대한 메커니즘 분석 및 임상 적용에 대한 심도 있는 연구를 통해서 췌장암 진행의 병태생리를 이해하는 데에 큰 도움을 줄 것이라고 생각한다.

Keywords: GV 1001, Gemcitabine, 췌장암이종이식동물모델, 췌장암

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