# The role of spleen for anti-tumor effect and tumor-infiltrating T lymphocytes in an orthotopic murine pancreatic cancer model

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Directed by Professor Chang Moo Kang

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**Ho Kyoung Hwang**

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This certifies that the Doctoral Dissertation of Ho Kyoung Hwang is approved.

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## ACKNOWLEDGEMENTS

Although I sometimes feel my way is so difficult during treating and performing complicated surgery for the patients with pancreatic and biliary cancer, I get the strength again from the warm greetings that patients give me. I realize that I do not do something for the patients, but that the patients are comforting me. It is perhaps the love for patients, the dedication of my teachers, and support of my family to overcome fear of pancreatic surgery and try to do my best for better treatment for patients. Therefore, I would like to express my gratitude to the professors who taught me about the difficult operation and my beloved Ye-on, Kyu-on, my lovely wife, Hee Ryung Lee, parents and parents-in-law first.

I was able to catch the concept of this paper thanks to Prof. Chang Moo Kang who first proposed the idea that preserving the spleen in patients with pancreatic cancer might help improve patient recovery and survival. I would like to express my sincere appreciation to Prof. Chang Moo Kang, who still works enthusiastically and conducts research. I am deeply grateful to Prof. Dong Sup Yoon who taught me everything about the pancreatic surgery so that I can still do

the surgery without any problems, but also Prof. Woo Jung Lee who does not hesitate to advise me on various difficulties and lead me to wisdom. I would also like to express my gratitude to all of my loving fellows, especially Sung Hwan Lee, who is helping me day and night and to my sincere colleague, Prof. Jae Keun Kim who shares my heart. Finally, I would like to thank Prof. Seungmin Bang, Prof. Yoo-Seok Yoon, Prof, Ja Seung Koo, and Prof. Sun Och Yoon for their generous advice, many helpful advices and guidance for this paper.

I hope that it will be a little bit helpful research in the treatment of patients suffering from pancreatic cancer.

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**ABSTRACT**

**The role of spleen for anti-tumor effect and tumor- infiltrating T lymphocytes in an orthotopic murine pancreatic cancer model**

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(Directed by Professor Chang Moo Kang)

**Background**: Splenectomy during cancer surgery might negatively influence on the oncologic outcomes of the patients. The purpose of this study is to investigate whether simultaneously splenectomy influences the tumor growth pattern in an orthotopic murine pancreatic cancer model.

**Materials and Methods**: Murine pancreatic cancer cells (PAN02) were subcutaneously injected into the flank of nude mouse. A small tumor fragment (3 $mm^2$), harvested from a subcutaneously injected nude mouse, was orthotopically implanted in the pancreas tail of C57/BL6 mouse without splenectomy (control group, n=15) or with simultaneous splenectomy (splenectomy group, n=15). Tumor growth patterns were analyzed by laparotomy at 21$^{st}$ day after surgery.

**Results**: Tumor rejection was observed in one mouse of control group. No tumor growth was found in 5 mice (33.3%) of control group and 1 mouse (6.7%) of splenectomy group ($p$=0.169). Tumor width and total tumor volume were significantly larger in splenectomy group ($p$=0.017; $p$=0.013, respectively). Peritoneal seeding was more frequently identified in the splenectomy group (11 (73.3%) *vs.* 4 (26.7%),

*p*=0.011). There were no differences in the number of liver and kidney metastasis between the two groups. The ratios of tumor-infiltrating $CD4^+$ to $FoxP3^+$ and $CD8^+$ to $FoxP3^+$ were significantly higher in the control group compared to the splenectomy group (8.2 ± 9.3 vs. 2.4 ± 1.5, *p*=0.046; 2.5 ± 1.4 vs. 1.5 ± 0.4, *p*=0.031, respectively).

**Conclusion**: Splenectomy enhanced tumor growth and peritoneal seeding in an orthotopic syngeneic murine pancreatic cancer mice model. The ramifications of these results are discussed for pancreatic cancer treatment.

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Key words: Pancreatic cancer, Spleen, Tumor-infiltrating lymphocyte, Splenectomy

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## I. INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a lethal gastrointestinal malignant disease with overall 5-year survival rate of less than 5%. Only about 15-20% of newly diagnosed patients are indicated for surgery and their 5-year survival rate around 15-20%[1,2]. Standard treatment modalities such as chemotherapy or chemo-radiation therapy have been ineffective for improving survival in pancreatic cancer patients[2]. Several clinical trials with targeted drugs also did not show significant survival improvement in advanced PDAC[3].

When we consider left-sided PDAC, distal pancreatectomy including splenectomy has been accepted as standard procedure. The reason why splenectomy should be included is that margin-negative resection and effective regional lymph node clearance are possible through splenectomy. If the tumor is located away from the splenic hilum and there is no evidence of nodal metastasis around spleen in the preoperative imaging studies, there is a need to reconsider to include splenectomy for routine procedure during distal pancreatectomy.

We previously reported that the actual splenic hilar lymph node metastasis rate in well-selected left-sided pancreatic cancer (small

tumor size, <3cm, and located away from splenic hilum) was very low enough that splenectomy-omitting radical distal pancreatectomy would be feasible through international multi-center survey[4]. In addition, spleen-preservation may provide positive effect for oncologic outcome considering the antitumor impact of spleen. In some clinical studies, splenectomy had a negative effect on cancer survival of patients with gastric and colon cancer[5-7]. Schwarz et al[8] reported that splenectomy had a negative influence on long-term survival independent of disease-related factor after pancreatectomy for PDAC. Although the effects of splenectomy on the antitumor immune system in vivo remain controversial, some reports found that the number of hepatic and lung metastases increased in splenectomized mice using colon cancer and liver tumor models[9-11]. In terms of PDAC, there is no in vivo study to demonstrate the spleen role for tumor growth or oncologic outcome.

The purpose of this study is to investigate whether splenectomy influences the tumor growth pattern, as well as host immunity, in an orthotopic syngeneic murine pancreatic cancer model.

## II. MATERIALS AND METHODS

### 1. Cell culture

The murine pancreatic cancer cell line, PAN02, was maintained in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen, Melbourne, Australia) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO). The cells were incubated at 37$^{o}$C in a humidified incubator of 5% CO2 in air. The cells were collected after trypsinization and stained with trypan blue (Sigma-Aldrich, St. Louis, MO). Only viable cells which excluded trypan blue were counted with a hemocytometer (Hausser Scientific, Horsham, PA).

### 2. Animals

Athymic nu/nu and C57/BL6 strain mice (AntiCancer Inc., San Diego, CA), 4– 6 weeks old, were used for subcutaneously tumor cells injection models and orthotopical tumor implant models respectively. Mice were kept in a barrier facility under HEPA filtration and fed with autoclaved laboratory rodent diet. All mouse surgical procedures were performed with the animals anesthetized by intramuscular injection of a 0.02 ml solution of 50% ketamine, 38% xylazine, and 12% acepromazine maleate. The animals were sacrificed at 21$^{st}$ day after surgry for investigating the tumor growth. Intravenous injection of ketamine mixture solution was used for euthanasia. To ensure death following injection, cervical dislocation was performed. All animal studies were conducted with an AntiCancer Institutional Animal Care and Use Committee (IACUC)-protocol specifically approved for this study and in accordance with the principles and procedures outlined in

the National Institute of Health Guide for the Care and Use of Animals under Assurance Number A3873-1.

**3. Subcutaneously tumor cell injection**

PAN02 cells were harvested by trypsinization and washed twice with phosphate buffered saline (PBS; Sigma Aldrich). The cells ($2x10^6$) were injected subcutaneously into the right and left flanks of nude mice within 30 min of harvesting. Subcutaneously injected tumors grew by about 20 mm at 6 weeks aftter implantation. The tumors were harvested for subsequent surgical orthotopic implantation (SOI).

**4. Orthotopic tumor implantation and simultaneously splenectomy**

The surgical orthotopic implantation (SOI) of tumor fragment was performed in C57/BL6 mice, as previously described[12-15]. The mice were divided into two groups according to simultaneously splenectomy or not (**Figure 1**). A small 6-10 mm transverse incision was made on the left flank of the mouse through the skin and peritoneum. The pancreas tail and spleen were exposed through this incision, and a single tumor fragment ($3mm^3$) retrieved from subcutaneous tumor of nude mouse was sutured to the tail of the pancreas using 7-0 nylon surgical sutures (Dermalon$^{TM}$, Covidien; Medtronic Inc., MN, USA). In control group, the pancreas tail and spleen were returned to the abdomen, and the incision was closed in one layer using 6-0 nylon surgical sutures (Dermalon$^{TM}$). In simultaneously splenectomy group, splenic artery and vein in splenic hilum and short gastric vessels communicating with spleinc upper pole were securely ligated with 7-0 nylons (Dermalon$^{TM}$). After spleen was removed from the pancreas tail,

a single tumor fragment was sutured to the pancreas tail (**Figure 2**). Fifteen mice were enrolled in each control and splenectomy group.

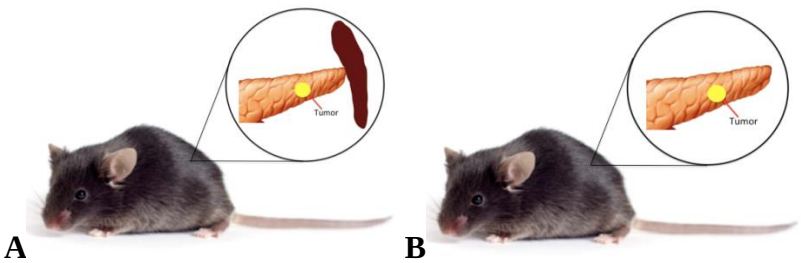


**Figure 1. Schema of experimental plan.** Small fragments (3 mm) of murine pancreatic cancer (PAN02) previously grown subcutaneously were orthotopically implanted in the pancreatic tail of C57/BL6 mice without splenectomy (**A**, control group) or with splenectomy (**B**, splenectomy group).


**Figure 2. Surgical procedures of surgical orthotopic implantation (SOI) of pancreatic cancer tumor fragments with simultaneous splenectomy.** A small 6-10 mm transverse incision was made on the left flank of the mouse through the skin and peritoneum. The pancreas tail and spleen were exposed through this incision (**a**, **b**). Splenic artery and vein in splenic hilum (white arrow) and short gastric vessels communicating with splenic upper pole (white arrow head) were securely ligated (**c**). After spleen was removed from the pancreas tail (**d**), a single tumor fragment (3 $mm^3$) was sutured to the pancreas tail using 7-0 nylon surgical sutures (white arrow head, **e**). On completion, the pancreas tail was returned to the abdomen, and the incision was closed in one layer using 6-0 nylon surgical sutures.

**5. Tumor growth pattern analysis**

Tumor growth patterns were analyzed by laparotomy. The animals were sacrificed 21$^{st}$ day after surgery. Tumor length and width were measured with calipers and tumor volume was calculated by the following formula: tumor volume = (length x width$^2$)/2. In addition, peritoneal cavity was carefully assessed for the evidence of peritoneal, hepatic, or other site metastases.

**6. Immunohistochemical staining and quantification of TILs subsets**

Immunohistochemical (IHC) staining for TILs subsets was performed as previously described except one mouse with tumor rejection in control group[16,17]. Briefly, paraffin-embedded tumor tissue sections at a thickness of 4-μm were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol. Antigen retrieval was performed in citrate buffer in a microwave oven. Endogenous peroxidase activity was blocked by incubating the tissue with 3% hydrogen peroxide in methanol for 5 min. The sections were incubated for 60 min at room temperature with primary monoclonal antibodies against cluster of differentiation (CD)4 (Cat. No. ab183685, 1:100, Abcam, Cambridge, UK), CD8 (Cat. No. ab203035, 1:100, Abcam, Cambridge, UK), and Foxp3 (Cat. No. ab20034, 1:100, Abcam, Cambridge, UK), which were used to identify helper T lymphocytes, cytotoxic T lymphocytes, and regulatory T lymphocyte (Treg), respectively. After washing the sections twice with 0.05 mol/L Tris-buffered saline with 0.2% Tween-20, the sections were incubated with horseradish peroxidase-conjugated secondary antibody (Dako EnVision® Detection system, Dako, Glostrup,

Denmark), followed by development with diaminobenzidine and counterstaining with hematoxylin.

IHC staining was quantified by experienced pathologist who was blinded to patient data. Three intense foci of staining in the tumor sections were selected and four high-power fields (magnification, x400) from each slide were selected for calculation of IHC staining results (**Figure 3**). Fields with necrosis or hemorrhage in the tumor portion were avoided.

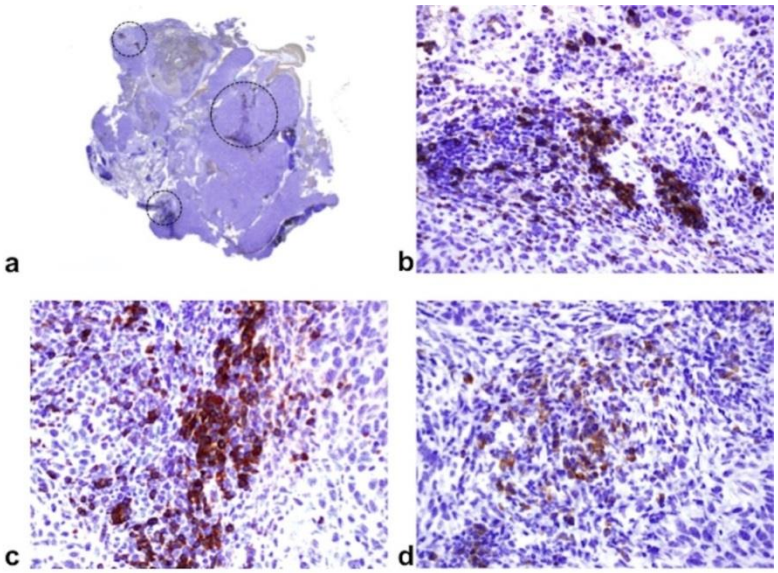


**Figure 3. Immunohistochemical staining of tumor-infiltrating T lymphocytes.** (a) After reviewing the staining results, three intense foci of staining were selected for cell count (magnification, x12). Immunohistochemical detections of (b) $CD4^+$ helper T lymphocytes, (c) $CD8^+$ cytotoxic T lymphocytes, and (d) forkhead box $P3^+$ regulatory T lymphocytes in consecutive sections were performed with high-power field (magnification, x400).

**7. Statistical analysis**

All statistical analyses were performed with SPSS 20.0 software (IBM, New York, NY, USA). Categorical variables were compared using $\chi^2$ or Fisher exact tests. Continuous variables are presented as mean ± standard deviation (SD) and the significance was determined using Student's *t*-test. Bar graph expressed mean value, and error bars show -SD. A *p*-value of 0.05 or less indicated statistical significance.

## III. RESULTS

### 1. Tumor growth patterns

Body weight at SOI and laparotomy was not different between two groups. In splenectomy group, one mouse was died at 19$^{st}$ after surgery. The mouse had severe ascites and multiple peritoneal seeding at laparotomy. In one mouse of control group, the implanted tumor could not be identified at pancreas tail and only suture material was observed. Peritoneal fat was attached around the surgical bed. We defined the tumor growth pattern as ‘tumor rejection’. If the size of implanted tumor was less than 5 mm, the mouse was defined as ‘no growth’. No tumor growth was observed in 5 mice (33.3%) of control group and 1 mouse (6.7%) of splenectomy group. There was no statistical difference (**Figure 4, Table1**).

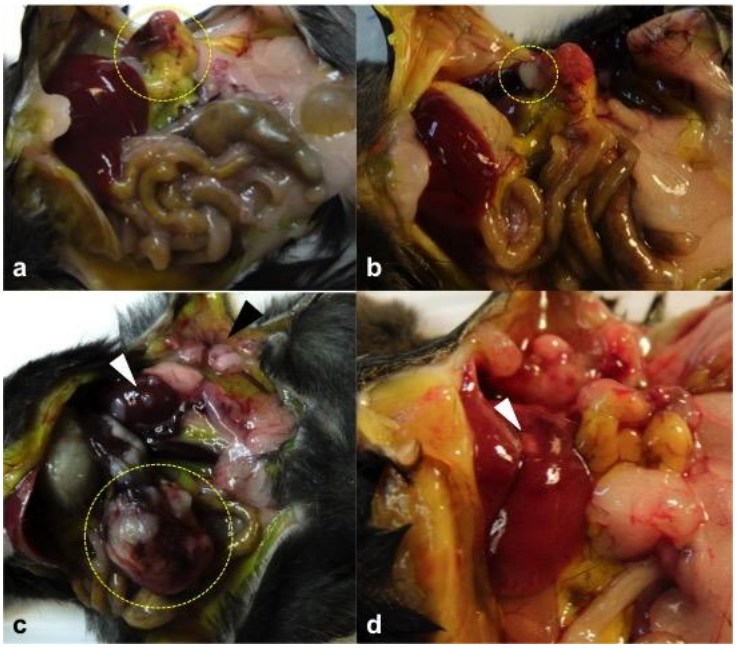


**Figure 4. Tumor growth patterns at laparotomy.** Tumor rejection was observed in one mouse (**a**). The implanted tumor was disappeared and only peritoneal fat was attached around surgical bed (**a**. yellow dotted circle). If the tumor size was less than 5mm, tumor growth pattern was defined as 'No growth' (**b**). Huge pancreatic tumor (**c**, yellow dotted circle), kidney metastasis (**c**, white arrow head) and peritoneal seeding (**c**, black arrow head) were observed. Liver metastasis was observed in one mouse (**d**, white arrow head).

**Table 1**. Tumor growth patterns according to splenectomy or not during surgical orthotopic tumor implantation

| | Control group (n=15) | Splenectomy group (n=15) | *p*-value |
|---|---|---|---|
| Body weight at SOI | 19.3 ± 2.6 | 18.18 ± 3.2 | 0.320 |
| Body weight at laparotomy | 21.8 ± 2.6 | 21.5 ± 3.4 | 0.797 |
| Postoperative death | | | 1.000 |
| No | 15 (100%) | 14 (93.3%) | |
| Yes | 0 | 1 (6.7%) | |
| Tumor rejection | | | 1.000 |
| No | 14 (93.3%) | 15 (100%) | |
| Yes | 1 (6.7%) | 0 | |
| Tumor growth | | | 0.169 |
| No | 5 (33.3%) | 1 (6.7%) | |
| Yes | 10 (66.7%) | 14 (93.3%) | |
| Tumor size (length), mm | 8.8 ± 5.0 | 11.3 ± 4.3 | 0.180 |
| Tumor size (width), mm | 6.2 ± 2.7 | 9.3 ± 3.8 | 0.017 |
| Tumor volume, $mm^3$ | 244.1 ± 239.5 | 697.9 ± 543.4 | 0.013 |
| Ascites | | | 0.068 |
| No | 10 (66.7%) | 5 (33.3%) | |
| Yes | 5 (33.3%) | 10 (66.7%) | |
| Peritoneal seeding | | | 0.011 |
| No | 11 (73.3%) | 4 (26.7%) | |
| Yes | 4 (26.7%) | 11 (73.3%) | |

| | | | |
|---|---|---|---|
| Liver metastasis | | | 1.000 |
| No | 14 (93.3%) | 15 (100%) | |
| Yes | 1 (6.7%) | 0 | |
| Kidney metastasis | | | 1.000 |
| No | 14 (93.3%) | 14 (93.3%) | |
| Yes | 1 (6.7%) | 1 (6.7%) | |

SOI: surgical orthotopic implantation

Tumor length was larger in the splenectomy group when compared to the control group, however there was no statistical difference ($p$=0.180). Tumor width and total tumor volume were significantly larger in splenectomy group ($p$=0.017; $p$=0.013, respectively) (**Table 1**, **Figure 5**).

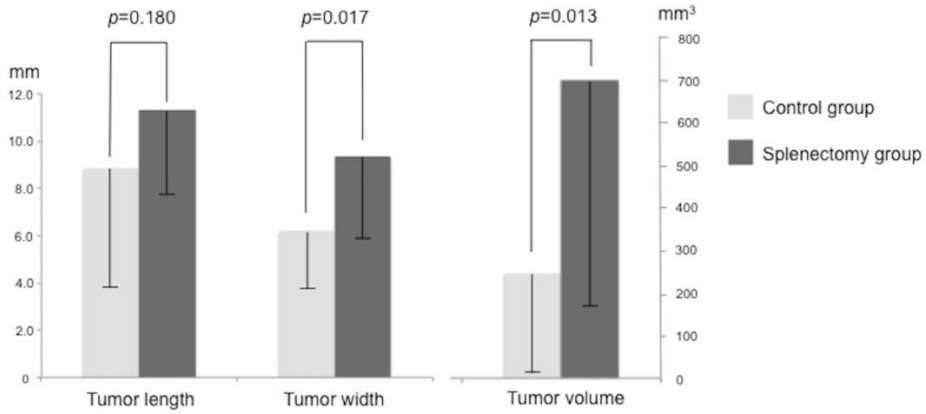


**Figure 5. Tumor size and volume in splenectomy and control groups.** Tumor width and total tumor volume were significantly larger in splenectomy group compared to control group.

**2. Tumor metastases patterns**

Severe ascites was observed in 5 (33.3%) mice of control group and 10 (66.7%) mice of splenectomy group with marginally significance ($p$=0.068). Peritoneal seeding was more frequently identified in the splenectomy group when compared to the control group (11 (73.3%) *vs.* 4 (26.7%), $p$=0.011). Liver metastasis was observed in only one mouse of control group. Kidney metastases were observed in one mouse of each group (**Table1**).

**3. Tumor-infiltrating T lymphocyte analysis**

The ratios of CD4+ to FoxP3+ and CD8+ to FoxP3+ were significantly higher in control group compared to splenectomy group (8.2 ± 9.3 vs. 2.4 ± 1.5, $p$=0.046; 2.5 ± 1.4 vs. 1.5 ± 0.4, $p$=0.031, respectively) (**Table 2**).

**Table 2**. Tumor infiltrating lymphocyte subsets count according to splenectomy or not during surgical orthotopic tumor implantation

| | **Control group (n=14)** | **Splenectomy group (n=15)** | ***p*-value** |
|---|---|---|---|
| **Absolute count** | | | |
| $CD3^+$ | 62.3 ± 57.5 | 82.6 ± 71.0 | 0.447 |
| $CD4^+$ | 91.5 ± 83.4 | 100.3 ± 76.2 | 0.790 |
| $CD8^+$ | 63.1 ± 66.7 | 81.6 ± 68.8 | 0.512 |
| Granzyme $B^+$ | 41.3 ± 43.3 | 73.7 ± 64.6 | 0.157 |
| $Foxp3^+$ | 24.9 ± 28.5 | 57.1 ± 55.9 | 0.084 |
| **Relative ratio** | | | |
| $CD3^+/Foxp3^+$ | 3.1 ± 2.9 | 1.6 ± 0.5 | 0.102 |
| $CD4^+/Foxp3^+$ | 8.2 ± 9.3 | 2.4 ± 1.5 | 0.046 |
| $CD8^+/Foxp3^+$ | 2.5 ± 1.4 | 1.5 ± 0.4 | 0.031 |
| Granzyme $B^+/Foxp3^+$ | 2.4 ± 1.8 | 1.5 ± 0.8 | 0.111 |

## IV. DISCUSSION

This study investigated the impact of splenectomy on tumor growth and host immunity in an orthotopic pancreatic cancer mouse model. The results showed that the tumor volume was significantly larger and peritoneal seeding was more frequent in the splenectomy group compared to the control group. The tumor growth pattern in the splenectomized mice was thus associated with more aggressive behavior.

Although the impact of splenectomy on experimental models with several kinds of cancer has been studied, the exact mechanism of spleen influencing cancer growth remains uncertain. Shiratori et al[11] and Imai et al[10] reported that the number of hepatic and lung metastases increased in splenectomized mice using colon cancer and liver tumor models respectively. They explained the mechanism of increasing of liver and pulmonary metastases as being due to decreased activity of NK cells after splenectomy. In these previous reports to demonstrate the impact of splenectomy on tumor growth, cancer cells were directly injected into the superior mesenteric vein or liver parenchyma to establish a model of liver metastasis of colon cancer. When the cancer cells are injected in a vein or into the liver parenchyma, there is chance of widespread cancer cell dissemination or intravasation into the portal vein or hepatic vein. With these experimental designs, cancer cells enter the circulation at the initial stage of tumor growth which is a different situation from natural tumor growth. In our study, we orthotopically implanted tumor fragments in the pancreas tail which is a very similar situation to the clinical setting of patients with left-sided

pancreatic cancer. In those cases, we can try to preserve the spleen in well-selected patients (for example, no evidence of tumor invasion to the spleen, no splenic hilum lymph node metastasis, small sized tumor etc.) during distal pancreatectomy.

Another possible mechanism for splenectomy effect for aggressive tumor behavior is that regulatory T cells may increase in some organs. Higashijima et al[9] reported that the number of hepatic metastases significantly increased in the splenectomy group compared to spleen-preserved group after colon cancer cells injected into spleen. They concluded that splenectomy enhanced hepatic metastasis through the increase of Foxp3 mRNA in the liver. Future studies will focus on the mechanism of increased metastases after splenectomy during pancreatectomy.

Our results showed that the relative ratios of $CD4^+$ helper T lymphocyte to $FoxP3^+$ Treg and $CD8^+$ cytotoxic T lymphocytes to $FoxP3^+$ Treg were significantly higher in control group compared to splenectomy group. We also previously reported the ratios of helper T cells or cytotoxic T cells to Treg were significantly related to patients' survival outcomes in the patients with gastric and pancreatic cancer[16,17]. Shang B et al[18] reported in their review that the high $FoxP3^+$ Tregs infiltration was significantly associated with shorter overall survival in the majority of solid tumors. Future experiments will measure other immune cells including myeloid derived suppressor cells, NK cells, M1 and M2 macrophages as well as time-course studies of the actual numbers of $CD4^+$ helper lymphocytes, $FoxP3^+$Treg, and $CD8^+$ cytotoxic T lymphocytes during pancreatectomy

When we consider the clinical setting, spleen-preserving distal pancreatectomy can be attempted at the time of cancer surgery in patients with PDAC. Therefore, the actual impact of splenectomy on tumor recurrence and oncologic outcomes will be further investigated with orthotopic models using other mouse pancreatic cancer cell lines as well as patient pancreatic cancer in orthotopic humanized mouse models. Future experiments will determine if there is a difference in tumor recurrence or survival depending on the spleen preservation or not during tumor resection in an orthotopic pancreatic cancer mouse model. Reimplantation of spleen fragments after pancreatectomy will also be done in the mouse models to determine if this can restore efficient antitumor mouse function. Clinical studies will also be performed to further understand the tumor immunological effects of splenectomy which will be measured by flow cytometry as well as immunohistochemistry.

## V. CONCLUSION

The present experiment, to our knowledge, is the first study to examine the effect of splenectomy on tumor growth and host immunity in orthotopic murine pancreatic cancer model. In conclusion, splenectomy enhanced tumor growth and peritoneal seeding in an orthotopic syngeneic murine pancreatic cancer implanted mouse model. The high ratio of tumor-infiltrating helper T or cytotoxic T lymphocytes to Treg seems to have important anti-tumor immunity in control group. The present results suggest that splenectomy be avoided during pancreatic resection for PDAC whenever possible.

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**ABSTRACT(IN KOREAN)**

## 비장이 췌장암의 동소이식모델에서 종양성장억제 및 종양침윤T림프구에 미치는 역할 규명

<지도교수 강 창 무>

연세대학교 대학원 의학과

황 호 경

**배경:** 암수술을 할 때 비장절제가 환자의 예후에 좋지 않은 영향을 미친다는 보고가 있다. 본 연구의 목적은 마우스 췌장암의 동소이식모델을 만들 때 비장을 함께 절제한 경우, 그렇지 않은 모델에 비해 종양의 성장 속도 및 전이여부에 차이가 있는지를 보고자 하며, 이러한 차이가 종양면역학적인 면에서 기인한 것인가를 종양 침윤 T 림프구를 조사하여 그 기전의 일부를 밝히는 데에 목적이 있다.

**방법:** 마우스 췌장암 세포주 (PAN02)를 누드마우스의 양쪽 등쪽 측면에 피하주입 시켰다. 누드마우스의 피하에서 종양이 어느 정도 자라면 이를 채취하여 작은 조각 (3 $mm^2$)으로 잘라서 C57/BL6 마우스의 췌장미부에 동소이식을 시행하였다. 이 때 비장절제 없이 동소이식한 그룹 (control group, n=15)과 비장절제술을 함께 시행한 그룹(splenectomy group, n=15)으로 나누어 실험을 진행하였다. 동소이식후 21일이 지난 시점에서 개복하여 종양의 성장 및 전이 여부를 관찰하였다.

**결과:** 동소이식한 마우스에서 종양이 자라지 않고 사라진 경우

(tumor rejection)가 control group의 한 마리 마우스에서 관찰이 되었다. 동소이식한 종양이 성장 없이 그대로 크기가 유지된 경우(No tumor growth)가 control group의 5마리(33.3%), splenectomy group의 한 마리(6.7%) 마우스에서 관찰이 되었고, 두 그룹간의 차이는 없었다 (p=0.169). 종양의 넓이와 전체 부피는 의미 있게 splenectomy group에서 크게 관찰이 되었다 (p=0.017, p=0.013). 복막전이 역시 splenectomy group에서 빈번이 관찰이 되었다 (11 (73.3%) vs. 4 (26.7%), p=0.011). 간전이와 신장전이 여부는 두 그룹간 차이는 없었다. 종양침윤 $CD4^+/Foxp3^+$ 비율과, $CD8^+/Foxp3^+$ 비율은 의미 있게 control group에서 높게 관찰이 되었다 (8.2 ± 9.3 vs. 2.4 ± 1.5, p=0.046; 2.5 ± 1.4 vs. 1.5 ± 0.4, p=0.031, respectively).

**결론:** 마우스 췌장암의 동소이식모델에서 비장절제술을 함께 시행했을 경우, 의미 있게 종양이 크게 성장하였고, 복막전이도 많이 발생하는 것을 관찰할 수 있었다. 이러한 결과는 실제 임상에서 췌장암 환자의 수술방침을 결정하는 데에 있어서 비장 보존 여부가 생존율 향상에 도움이 되는지에 대한 임상 연구의 기틀을 마련하였다고 볼 수 있다.

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핵심되는 말: 췌장암, 비장, 종양침윤 T림프구, 비장절제

# PUBLICATION LIST

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