**Master of Science**

# Immunosuppressive roles of IL-22 receptor-expressing myeloid cells in the progression of pancreatic ductal adenocarcinoma

# 췌관 선암종 진행 과정에서 증가하는 인터루킨-22 수용체 발현 골수성 세포의 면역 억제 기능

**The Graduate School**

**of the University of Ulsan**

**Department of Medical Science**

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# Immunosuppressive roles of IL-22 receptor-expressing myeloid cells in the progression of pancreatic ductal adenocarcinoma

This certifies that the master's thesis of Yoolim Sung is approved.

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## Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and fatal tumors worldwide. However, current biomarkers of PDAC remain inaccurate and imprecise for diagnosis. Here, we found that IL-10R2$^{+}$/IL-22R1$^{+}$ myeloid cells were increased in the blood and PDAC tissues of xenograft/orthotopic murine PDAC models. The increase in the population of IL-10R2$^{+}$/IL-22R1$^{+}$ myeloid cells in the blood of the xenograft murine model was dependent on the tumor size, which suggests that IL-10R2$^{+}$/IL-22R1$^{+}$ myeloid cells could be a liquid biopsy marker. In the orthotopic model, the population of IL-10R2$^{+}$/IL-22R1$^{+}$ myeloid cells increased in PDAC cell line injected group. Moreover, the population of IL-10R2$^{+}$/IL-22R1$^{+}$ myeloid cells also increased on day 7 after the injection, which is considered the early stage in the model. These results indicate that IL-10R2$^{+}$/IL-22R1$^{+}$ myeloid cells could be a biomarker of early PDAC. Furthermore, IL-10R2$^{+}$/IL-22R1$^{+}$ myeloid cells showed immunosuppressive effects. Notably, T cells co-cultured with IL-10R2$^{+}$/IL-22R1$^{+}$ myeloid cells showed decreased proliferation rate and cytotoxicity. Induction of differentiation into myeloid-derived suppressor cells increased the expression of IL-10R2 and IL-22R1 in human blood. Additionally, T cells cocultured with IL-10R2$^{+}$/IL-22R1$^{+}$ myeloid cells exhibited diminished proliferation. Therefore, IL-10R2$^{+}$/IL-22R1$^{+}$ myeloid cells could be a liquid biopsy marker or early-stage biomarker of PDAC and could possess immunosuppressive features.



Keywords : PDAC, biomarker, IL-22R, myeloid cells, immunosuppressive TME

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# Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant tumors worldwide, ranking seventh among all cancers in terms of mortality rate[1]. Owing to its subtle or nonspecific markers, early diagnosis of PDAC remains difficult. Studies have shown that patients diagnosed with early-stage PDAC have higher survival rates than do those diagnosed with late-stage disease, although the majority of patients are diagnosed with stage III/IV PDAC, resulting in high mortality rates[2,3]. The survival rate of patients with PDAC largely depends on their diagnosis stage[4]. Recent research has been dedicated to uncovering novel markers to facilitate early detection and improve the therapeutic management of PDAC.

The tumor microenvironment (TME) of PDAC is nonimmunogenic, making it resistant to therapy[5]. As the tumor progresses, PDAC cells modulate the host immune cells in the TME to establish an immunosuppressive TME[6]. PDAC recruits immunosuppressive cells, such as tumor-associated macrophages, tumor-associated neutrophils, myeloid-derived suppressor cells (MDSCs), and regulatory T cells by secreting cytokines and chemokines. In particular, MDSCs alleviate the proliferation and cytotoxicity of cytotoxic T cells ($CD8^+$ T cells) to establish an immunosuppressive TME[5-7].

Liquid biopsies are the newly emerging technology in precision medicine that has been applied for the early detection and monitoring of minimal residual disease or acquired resistance to therapies in solid cancers[8]. Such biopsies are performed by isolating tumor-derived components, such as circulating tumor cells, circulating tumor DNA, tumor extracellular vesicles, and tumor-associated immune cells, from the body fluids of cancer patients, and analyzing the biomarkers contained within them[9,10].

Interleukin-22 (IL-22), a member of the interleukin-10 (IL-10) cytokine family[11], transduces a signal through the IL-22 receptor (IL-22R) composed of a heterodimer of IL-10R2 and IL-22R1[12]. IL-22 has been known to be involved in the homeostasis and repair of fibroblasts and nonhematopoietic epithelial cells in the lungs, livers, and intestines[13,14,15]. However, the roles of IL-22 and IL-22R in PDAC TME have been poorly investigated.

Research has shown that IL-22 expression was significantly higher in PDAC tissues than in healthy pancreas tissues[16,17]. We hypothesized that IL-22-recognizing immune cells could be affected by this elevation in IL-22 in the PDAC TME and could be involved in further tumor progression. Therefore, to clarify whether IL-22R-expressing immune cells are affected during PDAC progression, we developed a xenograft/orthotopic murine PDAC model and analyzed the population of IL-22R-expressing immune cells during tumor progression. Notably, we found that IL-22R-expressing myeloid cells increase in the

blood and tumor tissues even in the early phases of PDAC progression. Additionally, we analyzed the effects of IL-22R-expressing myeloid cells on immune activation in murine and human cell culture systems and found that they can suppress T cell proliferation and cytotoxicity in co-culture experiments. Consequently, our results suggest that IL-22R-expressing myeloid cells can function as a liquid biopsy marker or early marker of PDAC and as an immunosuppressive player in TME.

## Materials and methods

### Animal models

All animal experiments were approved by the Animal Care and Use Committee of the Asan Institute for Life Science and Yonsei University College of Medicine and conducted following the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, no. 85-23, 1996) and ARRIVE Essential 10 guidelines[18]. BALB/c nude mice (8-10-week-old) were obtained from Charles River Laboratory. C57BL/6 mice (6-7-week-old) were obtained from the Jackson Laboratory. Mice were maintained in a specific pathogen-free environment. Before each experiment, mice were acclimated for at least 1 week and randomly assigned to experimental groups. Murine PDAC models were established as previously described[19,20]. Briefly, mice were separately placed into an anesthetizing chamber and anesthetized with 2%-3% isoflurane in 100% oxygen. For the xenograft murine model, the $1 \times 10^7$ PANC-1 cells were injected subcutaneously into the back skin of BALB/c nude mice. For the orthotopic murine PDAC model, $2 \times 10^6$ PANC-1/AsPC cells and $1 \times 10^6$ Pan02 cells were orthotopically implanted into the pancreas of BALB/c nude mice and C57BL/6 mice respectively.

### Flow cytometric analysis and sorting

Mouse peripheral blood cells were treated with red blood cell lysis buffer (ThermoFisher, 00-4333-57, USA) two times and used for further analysis. To isolate tumor-infiltrated lymphocytes, tumor tissues were treated with collagenase (Sigma-Aldrich, C7657, USA) and dispase (Sigma-Aldrich, D4818, USA) (human PDAC tissues) or collagenase and Dnase1 (Roche, 10104159001, Swiss) (murine PDAC model tumor tissues). Cells were further stained with Fc blocker and antibodies at 4℃. TruStain FcX antimouse CD16/32 antibody (Biolegend, 101320, USA), PE antimouse IL-22R1 antibody (R&D Systems, FAB42941P, USA), APC antimouse IL-10R2 antibody (R&D Systems, FAB53681A, USA), FITC antimouse CD3 antibody (Biolegend, 100306, USA), PE/Cy7 antimouse CD8 antibody (Biolegend, 100722, USA), Percp/Cyanine5.5 antimouse CD45 antibody (Invitrogen, 45-0451-82, USA), PE antimouse Granzyme B antibody (Biolegend, 372207, USA), Percp/Cyanine5.5 antihuman IL-22R1 antibody (R&D, FAB2770C, USA), PE antihuman IL-10R2 antibody (Biolegend, 308804, USA), APC/Cyanine7 antihuman CD3 antibody (Biolegend, 344817, USA), Pacific Blue antihuman CD8 antibody (Biolegend, 344717, USA) were used for staining.

The Zombie Aqua Fixable Viability Kit (Biolegend, 423101, USA) was used to identify live cells. The

cells were analyzed using a FACS Canto II cytometer or sorted using a BD FACS Aria III cell sorter. FlowJo (version 10) was used for data analysis.

**Cell lines and cell cultures**

Human pancreatic cancer cell lines (PANC-1 and AsPC) and murine pancreatic cancer cell line (Pan02 cells) were purchased from ATCC. Cells were cultured in RPMI 1640 media with 20 mM HEPES and L-glutamine without sodium bicarbonate supplemented (Gibco, 724000120, USA) with 10% heat-inactivated fetal bovine serum (Gibco, 16000044, USA) and 1% penicillin-streptomycin (Gibco, 15070063, USA) at 37℃ in a 5% $CO_2$ humidified environment.

Human peripheral blood mononuclear cells (PBMCs) (Stem Cells Technology, Canada) were cultured with granulocyte-macrophage colony-stimulating factor (GM-CSF) (PeproTech, AF-300-03, USA) and interleukin-6 (IL-6) (PeproTech, AF-200-06, USA) at a concentration of 20 ng/mL each for 4 days to generate human MDSC cultures, following previously described protocols[21,22]. Human PBMC cells cultured with GM-CSF (20 ng/mL) alone were used as functional control.

**Immune suppression assay**

For murine T cell preparation, 2 μg/mL of antiCD3 antibody was coated on a 96-well round bottom plate at 4℃ overnight. T cells were then enriched using a mouse T cell enrichment kit (Stem Cells Technology, Canada) from the lymph nodes and spleen of C57BL/6 mice. Enriched T cells were stained with CFSE (2 μM) (Invitrogen, C34554, USA). CFSE-labeled T cells were co-cultured with sorted murine myeloid cells at a 20:1 ratio in the presence of antiCD28 antibody (2 μg/mL). Cells were harvested after 3 days and analyzed for CD3, CD8, CFSE, and granzyme B expression using flow cytometry. For human cells, T cells were generated by culturing PBMCs with 10 ng/mL of IL-2 (Gibco, PHC0026, USA) for 4 days. After labeling the cells with 2 μM CSFE, they were co-cultured with sorted myeloid cells at a 20:1 ratio in a 96-well round bottom plate. Following a 3-day incubation, the cells were harvested, stained with an antiCD8 antibody, and analyzed for CFSE levels using flow cytometry.

## Results

### IL-10R2$^+$/IL-22R1$^+$ myeloid cells as a liquid biopsy marker of PDAC progression in a murine PDAC model

To identify whether IL-22R-expressing myeloid cells can be a liquid biopsy marker of PDAC, we established a xenograft murine PDAC model by injecting the human PDAC cell line PANC-1 into BALB/C nude mice and analyzed the percentage of IL-22R-expressing myeloid cells in the blood (Fig. 1A). The population of IL-10R2$^+$CD45$^+$ and IL-22R1$^+$CD45$^+$ cells increased depending on tumor volume (Fig. 1B). The number of IL-10R2$^+$ and IL-22R1$^+$ myeloid cells were higher in the PANC-1 xenografted group compared to PBS injection group (Fig. 1C). Therefore, IL-10R2$^+$ and IL-22R1$^+$ myeloid cells could be a novel liquid biopsy marker in PBMCs of a xenograft murine PDAC model.

### Enrichment of tumor-infiltrating IL-22R expressing myeloid cells in the PDAC TME

An increase in the population of IL-22R-expressing myeloid cells in the blood could indicate an increase in IL-22R-expressing myeloid cells in the PDAC TME. To mimic the human PDAC TME, we established a xenograft murine PDAC model by orthotopically injecting PANC-1 cells or AsPC cells into BALB/C nude mice (Fig. 2A). Notably, an increase in the number of IL-10R2$^+$CD45$^+$ cells and IL-22R1$^+$CD45$^+$ cells were noted in tumor tissues 14 and 21 days after injection, which can be considered the middle and late stages of tumor progression, respectively (Fig. 2B). This result suggests that IL-10R2$^+$ -or IL-22R1$^+$- expressing myeloid cells could be a biomarker of PDAC.

### IL-10R2$^+$IL-22R1$^+$ myeloid cells as a marker of early PDAC

To investigate whether IL-22R-expressing myeloid cells were enriched in the early stages of PDAC, immune cells in tumor tissues were analyzed on day 7, which is considered the early stage of xenograft murine PDAC model in Figure 2(Fig.3A). IL-10R2$^+$IL-22R1$^-$ and IL-10R2$^+$IL-22R1$^+$ cells were significantly enriched in tumor tissues on day 7. However, IL-10R2$^-$IL-22R1$^+$ cells did not significantly increase after day 7 since the injection (Fig.3B). Therefore, IL-10R2$^+$/IL-22R1$^+$ myeloid cells can be an early biomarker of PDAC.

**Immunosuppressive features of IL-10R2$^+$IL-22R1$^+$ myeloid cells *in vitro***

Immune cells in tumors can modulate the TME toward immunostimulatory or immunosuppressive conditions[23]. IL-10R2$^+$ and IL-22R1$^+$ myeloid cells were abundant in the TME of the murine PDAC model (Fig. 1, and Fig. 2). Thus, these immune cells are suggested to be involved in altering the TME of the orthotopic murine PDAC model. To examine the immunomodulatory features of IL-10R2$^+$IL-22R1$^+$ myeloid cells, we isolated myeloid cells from the spleen of the orthotopic murine PDAC model 3 days after tumor injection. We sorted them into IL-10R2$^-$IL-22R1$^-$, IL-10R2$^+$IL-22R1$^-$, IL-10R2$^-$IL-22R1$^+$, and IL-10R2$^+$IL-22R1$^+$ cells (Fig. 4A). These cells were then co-cultured with CFSE-labeled T cells in the presence of stimulation with antiCD3 and antiCD28 antibodies. After 3 days of co-culture, the proliferation and cytotoxicity of CD8$^+$ T cells were significantly reduced when co-cultured with IL-10R2$^+$IL-22R1$^-$ or IL-10R2$^+$IL-22R1$^+$ myeloid cells (Fig. 4B). This result indicates that IL-22R-expressing myeloid cells could have immunosuppressive features in the PDAC TME.

**IL-10R2$^+$IL-22R1$^+$ myeloid cells were increased and had immunosuppressive features in human MDSC culture**

After evaluating the immunosuppressive roles of IL-22R-expressing myeloid cells from the murine PDAC model, we examined whether IL-22R-expressing myeloid cells have immunosuppressive functions in a human cell culture system. MDSC is one of the representative myeloid cells with immune suppressive features in the TME[39] and can be induced by culture with GM-CSF and IL-6 *in vitro*[40]. Therefore, we first examined the expression of IL-22R in this MDSC culture. The population of IL-10R2$^+$IL-22R1$^+$ myeloid cells was increased in the MDSC culture when compared to control (Fig.5A). Accordingly, we sorted IL-10R2$^+$IL-22R1$^-$, IL-10R2$^+$IL-22R1$^+$, and IL-10R2$^-$IL-22R1$^-$ cells from the MDSC culture, and conducted the immunosuppression assay by co-culturing with CFSE-labeled T cells in presence of IL-2. Notably, T cells co-culture with IL-10R2$^+$IL-22R1$^-$ and IL-10R2$^+$IL-22R1$^+$ myeloid cells showed a decline in proliferation compared to that with inflammatory monocytes. However, T cells cocultured with IL-10R2$^-$IL-22R1$^-$ myeloid cells exhibited increased proliferation than those with inflammatory monocytes (Fig.5B). Therefore, IL-22R-expressing myeloid cells could potentially create an immunosuppressive TME in human PDAC tissue.

## Discussion

Current circulating biomarkers of PDAC, such as CA19-9, lack the necessary sensitivity and specificity for effective diagnosis[24]. Despite extensive research, specific biomarkers for PDAC suitable for clinical use have yet to be established[3,25,26]. Therefore, establishing noninvasive methods from the early detection of cancer is essential for improving survival outcomes among PDAC patients.

IL-22 can be an immunostimulatory or immunosuppressive cytokine depending on the tissue or cancer type[27]. The anti-inflammatory roles of IL-22 in epithelial cell layers mainly include maintaining the barriers in the skin and tissue of respiratory or digestive systems, rather than directly affecting immune cells[28]. However, we showed that IL-22 could directly affect myeloid cells through IL-22R and create immunosuppressive features in the PDAC TME and the blood (Figs.1-5).

In the current study, we found that IL-10R2$^+$/IL-22R1$^+$ myeloid cells could be a promising novel liquid biopsy marker for PDAC patients. IL-10R2$^+$/IL-22R1$^+$ myeloid cells in the blood of the xenograft murine PDAC model were increased depending on PDAC tumor size. Also, the number of infiltrated IL-22R-expressing myeloid cells in the blood (Fig. 1). However, changes in the population of these cells need to be analyzed in the early stages of PDAC.

Nonetheless, further studies are needed to determine how IL-22R-expressing myeloid cells infiltrated the PDAC TME. Several factors can be involved in the accumulation and recruitment of MDSCs. Chemokines such as CXCL5, CXCL6, CCL2, CCL3, and CCL4 have been found to recruit MDSCs to the tumor tissue[29]. TME-derived hypoxia is a critical factor stimulating the recruitment of MDSCs. However, research has shown that IL-22 from ILC3 could recruit MDSCs in the TME[30]. Hence, IL-22 could possibly recruit MDSCs to the TME. Additionally, our data was limited in the roles of IL-22R-expressing myeloid cells in the murine PDAC model and human cell culture *in vitro*. As such, more research is needed to examine the sensitivity and specificity of IL-22R-expressing myeloid cells as a biomarker in PDAC patient samples.

Our findings showed that IL-22R-expressing myeloid cells had immunosuppressive functions *in vitro* (Fig. 4). MDSCs can inhibit the activation of T cells through several mechanisms[31]. First, MDSCs absorb cystine and do not release it, thereby keeping T cells inactivated[32]. Additionally, MDSCs produce adenosine through CD39 or CD73 to suppress the function of T cells[33]. Lastly, MDSCs can express PD-L1 to stimulate T cells to express PD-1[34]. These are well-known mechanisms by which MDSCs facilitate tumor immune evasion by repressing T cell function in the TME[28]. In our future study, will discuss the mechanism by which IL-22R-expressing myeloid cells suppress T cell function.

The current study found that IL-22R-expressing myeloid cells displayed immunosuppressive function in human PBMC culture system (Fig. 5). The immunosuppressive function of IL-22R-expressing myeloid cells has to be shown in human PDAC tissue. Also, we need to explore whether IL-22R is responsible for the immunosuppressive function of these cells or whether IL-22R is only a marker for immunosuppressive cells without functional roles. IL-22R can regulate JAK/STAT, MAPK, and PI3K/AKT pathways[12,35-38]. At first, activated JAK1 and TYK2 can stimulate the generation and function of MDSC through the phosphorylation of STAT3[39]. Furthermore, MAPK signaling can activate the ERK or p38 pathway, which plays a critical role in the differentiation and function of MDSCs[40]. The PI3K/AKT pathway stimulates the survival and function of MDSC and upregulates PD-L1, which is the immune checkpoint ligand[41,42]. Therefore, these pathways could be the mechanisms underlying the immunosuppressive function of IL-22/IL-22R axis expression.

In conclusion, our findings suggest that IL-22R-expressing myeloid cells could potentially be a liquid biopsy marker or an early biomarker of PDAC and can be used as a target for cancer treatment based on its immunosuppressive functions.

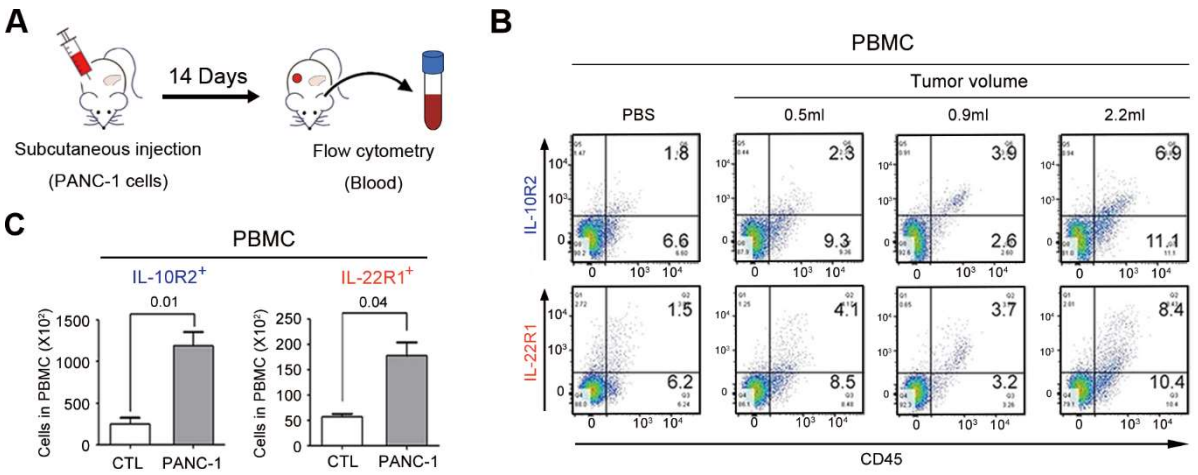


**Fig 1. Increased circulating IL-10R2$^+$/IL-22R1$^+$ myeloid cells in an orthotopic murine PDAC model**

(A) PANC-1 cells ($1 \times 10^7$ cells/mouse) were subcutaneously injected in BALB/c nude mice to establish a murine model. After 14 days of injection, the IL-10R2$^+$CD45$^+$ and IL-22R1$^+$CD45$^+$ cell populations in the blood were evaluated with flow cytometry. (B) Representative images of IL-10R2$^+$CD45$^+$ and IL-22R1$^+$CD45$^+$ cell populations in the blood were shown depending on tumor mass size. (C) Cell number of IL-10R2$^+$/IL-22R1$^+$ cells were quantified (means and standard deviation (SD)s, and *p*-value (Mann-Whitney U test) were shown).

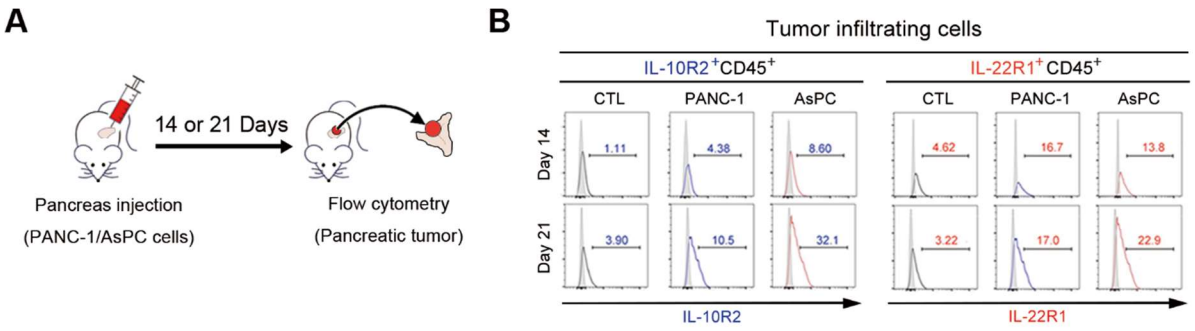


**Fig 2. Enrichment of IL-10R2$^+$/IL-22R1$^+$ myeloid cells in tumor tissues of orthotopic murine PDAC model during pancreatic tumor progression**

(A) An orthotopic murine PDAC model was established by PANC-1 or AsPC cell injection ($2 \times 10^6$ cells/mouse) respectively into the pancreas. (B) After 14 days or 21 days of injection, IL-10R2$^+$CD45$^+$ and IL-22R1$^+$CD45$^+$ cell populations in tumor tissue were evaluated with flow cytometry (means and standard deviation (SD)s, and *p*-value (Mann-Whitney U test) were shown).

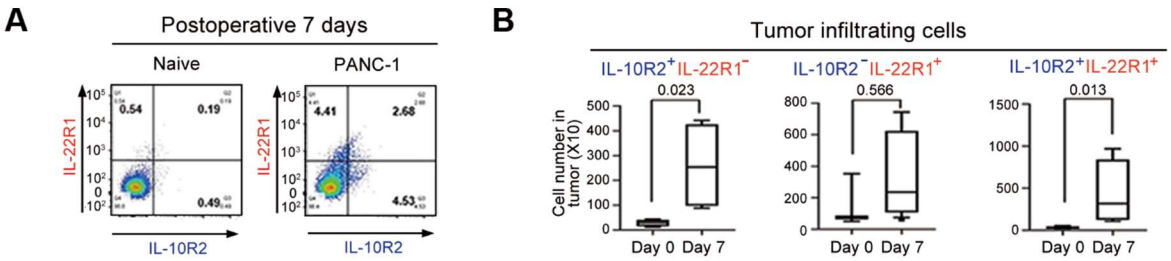


**Fig 3. Increased infiltration of IL-10R2$^{+}$IL-22R1$^{+}$ myeloid cells in an early-stage PDAC TME**

(A) As an early time-point in the same model of Fig.2, 7 days after the injection, the IL-10R2$^{+}$IL-22R1$^{+}$ cell populations in tissues of the control group and the PANC-1 injected group were measured with flow cytometry. (B) IL-10R2$^{+}$IL-22R1$^{-}$, IL-10R2$^{-}$IL-22R1$^{+}$, and IL-10R2$^{+}$IL-22R1$^{+}$ cell populations were quantified by cell number (means and standard deviation (SD)s, and *p*-value (Mann-Whitney U test) were shown).

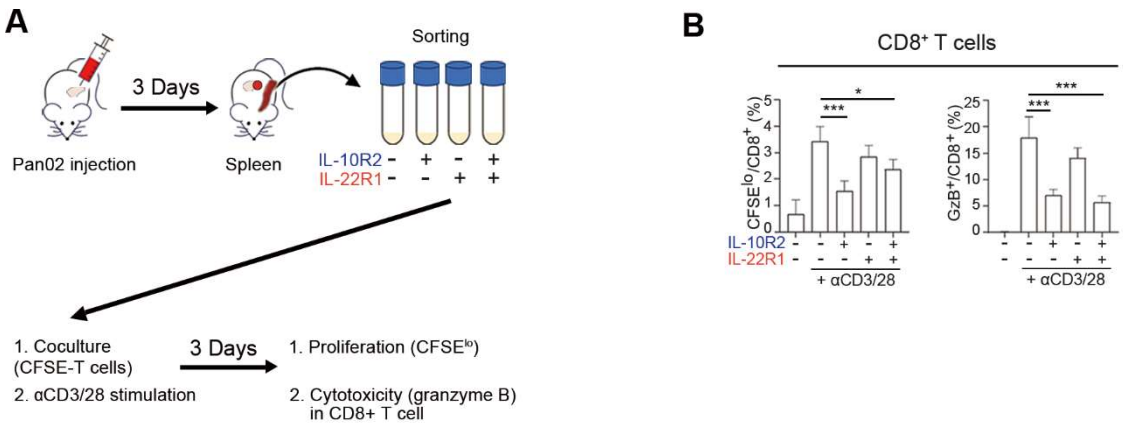


**Figure 4. Immunosuppressive functions of IL-10R2$^{+}$IL-22R1$^{+}$ myeloid cells from the orthotopic murine PDAC model**

(A) Scheme of immune suppression assays was shown. Spleens were harvested from the orthotopic murine PDAC model (C57BL/6 mice) and the cells were sorted by IL-10R2/IL-22R1 expression profile, then, sorted cells were co-cultured with CFSE-labeled T cells at a 1:20 ratio. After 3 days of co-culture, cells were analyzed by proliferation and cytotoxicity assay. (B) Proliferation (CFSE$^{lo}$) and cytotoxicity (Granzyme B (GzB$^{+}$)) of CD8$^{+}$ T cells were evaluated with flow cytometry. (Means and SDs were shown. *;$p$<0.05,**;$p$<0.01,***;$p$<0.001 by students' t-test)

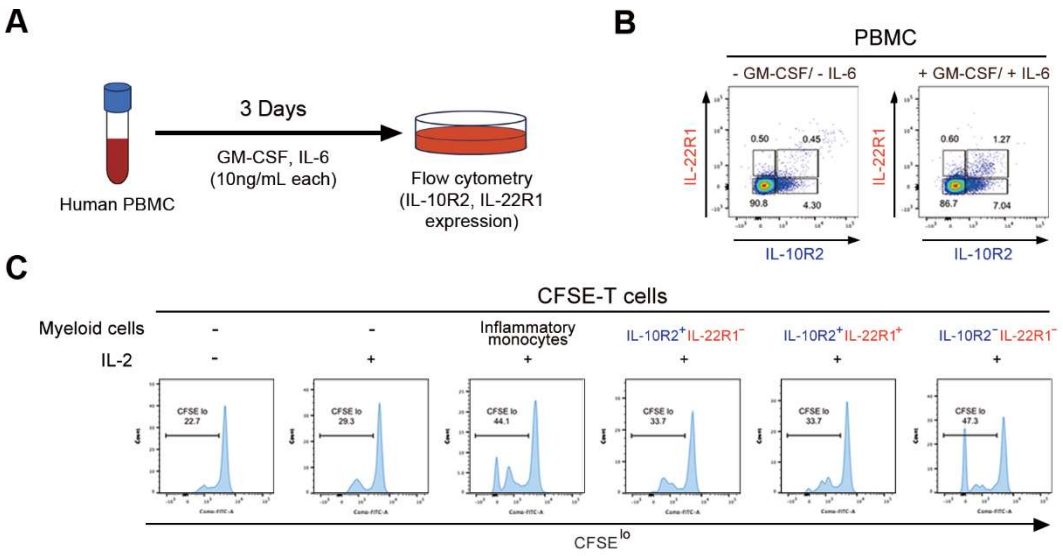


**Fig 5. The immunosuppressive function of IL-10R2$^{+}$IL-22R1$^{+}$ cells from human MDSC culture**

(A) Human PBMCs were treated with GM-CSF and IL-6 to induce MDSC, and IL-10R2/IL-22R1 expression was analyzed. (B) The IL-10R2 and IL-22R1 expressions in myeloid cells were measured by flow cytometry. (C) The proliferation (CFSE$^{lo}$) of T cell was evaluated in only T cell culture condition or co-culture with inflammatory monocytes, IL-10R2$^{+}$IL22R1$^{-}$, IL-10R2$^{+}$IL22R1$^{+}$, or IL-10R2$^{-}$IL22R1$^{-}$ cells in the presence of IL-2.

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## 국문 요약

췌관 선암종(PDAC)은 전 세계적으로 사망률 7위에 이르는 치명적인 종양 중 하나이다. 그러나 현재 PDAC의 생체표지자는 진단을 하기에 민감도와 정확도가 떨어진다. 본 연구에서는 이종 이식 또는 동종 이식 마우스 PDAC 모델의 혈액 및 PDAC 조직에서 IL-10R2$^+$/IL-22R1$^+$ 골수성 세포가 증가했음을 확인하였다. 이종 이식 마우스 모델의 혈액에서 IL-10R2$^+$/IL-22R1$^+$ 골수성 세포의 비율은 종양 크기에 따라 증가하였으며, 이는 IL-10R2$^+$/IL-22R1$^+$ 골수 세포가 액체 생검 표지자가 될 수 있음을 시사한다. 동종 이식 모델에서 IL-10R2$^+$/IL-22R1$^+$ 골수성 세포의 비율은 PDAC 세포주를 주사한 개체들에서 증가하였다. 또한, 동일 모델의 초기 단계로 간주되는 7일째의 PDAC 조직에서도 I IL-10R2$^+$IL-22R1$^+$ 골수성 세포의 비율이 증가하였다. 이러한 결과는 IL-10R2$^+$/IL-22R1$^+$ 골수성 세포가 초기 PDAC의 생체표지자가 될 수 있음을 보여준다. 또한 마우스 모델에서 IL-10R2$^+$IL-22R1$^+$ 골수성 세포와 공동 배양한 T 세포의 증식률과 세포독성이 감소함을 통하여 IL-10R2$^+$IL-22R1$^+$ 골수성 세포의 면역 억제 효과를 확인하였다. 골수 유래 면역 억제 세포로의 분화 유도는 성인의 혈액에서 IL-22R1과 IL-10R2의 발현을 증가시켰다. 성인의 IL-10R2$^+$IL-22R1$^+$골수성 세포와 공동 배양된 T 세포의 증식률이 감소됨을 확인하였다. 따라서 IL-10R2$^+$IL-22R1$^+$ 골수성 세포는 PDAC의 초기 생체 표지자 혹은 액체 생검표지자가 될 수 있으며, 면역 억제 기능을 가짐으로써 암 치료의 표적으로 이용될 수 있음을 확인하였다.