Circulating IL-10R2+ myeloid cells in peripheral blood are an early diagnostic marker for pancreatic ductal adenocarcinoma

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1. Introduction

●The immune system plays a crucial role in both the positive and negative regulation of tumor development and progression. However, about pancreatic ductal adenocarcinoma (PDAC), it is a poorly immunogenic tumor, and the exact mechanisms for the immunoresistance have not fully understood.

●The purpose of this study is to identified PDAC-specific bone marrow-derived immune cells from the patient's blood, determine surface marker analysis, and investigate the functional roles of these cells

2. Methods

● Human case-control study: The subjects who have diagnosed as PDAC, chronic pancreatitis (CP), bile duct cancer (BDC), lung cancer, and colon cancer were included.

● Flow cytometry & single cell RNA-sequencing (scRNA-seq) with patient's blood: From the whole blood, mononuclear cells were separated from the plasma and analyzed cell surface markers and transcriptomes by flow cytometry (FACS) and single-cell RNA-sequencing technique (RNA-Seq).

●Animal model for cancer induction (syngeneic graft and xenograft): Mice were placed individually in an anesthetizing chamber and anesthesia was induced with 2~3% isoflurane in 100% oxygen. 2×10^6 tumor cells were orthotopically implanted into the mouse pancreas. On the scheduled date, mice were sacrificed.

3. Result

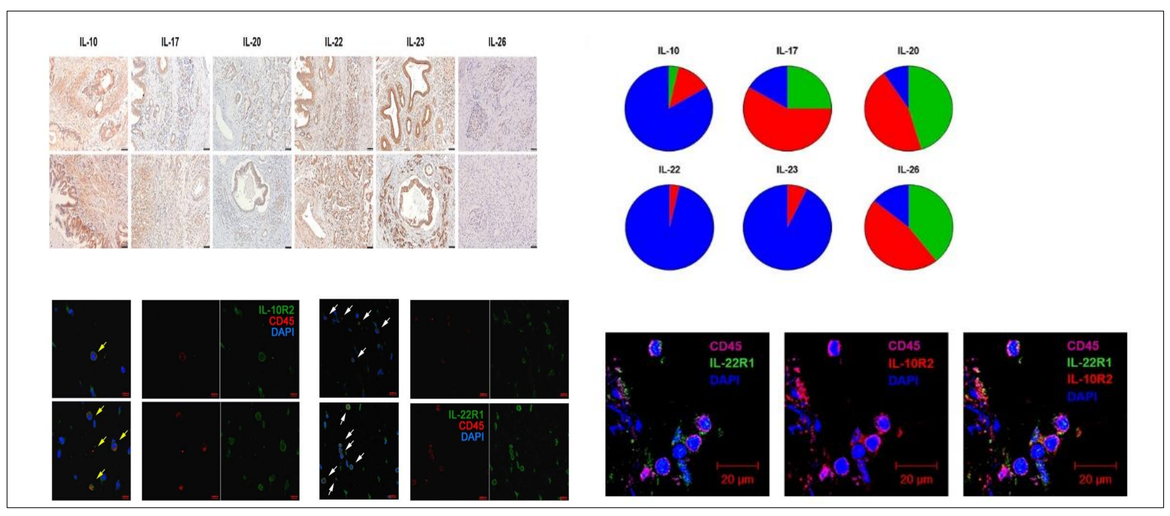


Figure 1. Characterization of cytokines upregulated in PDAC. By immunofluorescent staining of the pancreatic ductal adenocarcinoma TMA block, we confirmed the tumor-infiltrating CD45 (Leukocyte common antigen)+ immune cells co-express the IL -22R1 and IL -10R2.

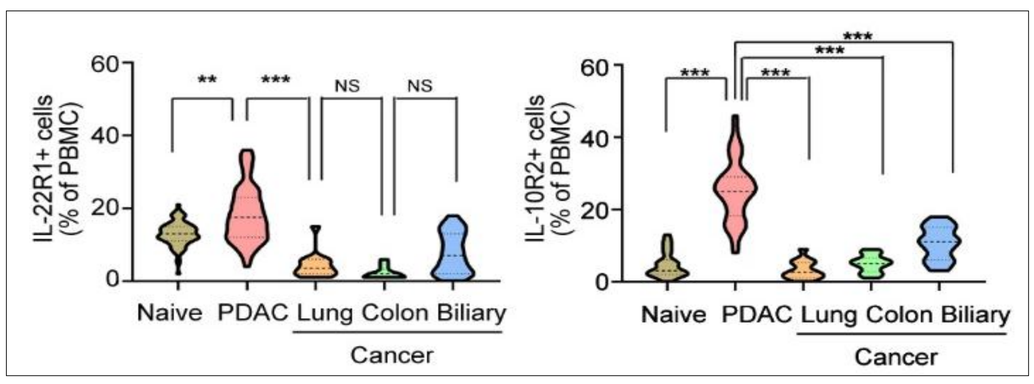


Figure 2. Census for circulating IL-10R2+ or IL-22R1+ PBMC between solid cancer patients. To verify these two markers are specific to Pancreatic cancer, we compare the serological value of the marker with that of naïve and other tumors using FACS. FACS data show IL-22R1 and 10R2 was significantly high in PDAC comparing the naïve and other types of cancer.

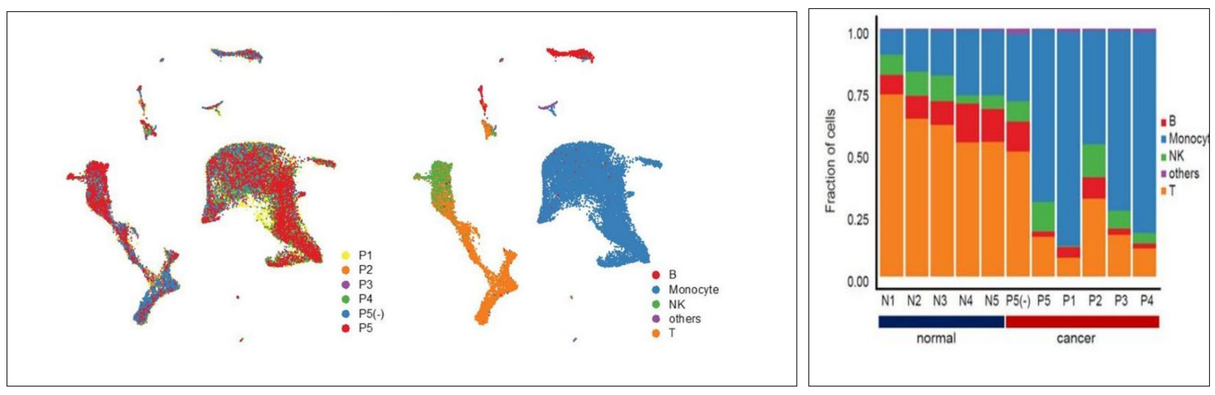


Figure 3. IL-10R2+ PBMC scRNA-seq . Total 24,819 cells passed our stringent quality control and showed different pattern of transcriptome between naïve and PDAC. Compared to IL-10R2- cells, the most prominent differences in IL-10R2+ cells were an expansion of monocytes/macrophages and a reduction in B and T cells .

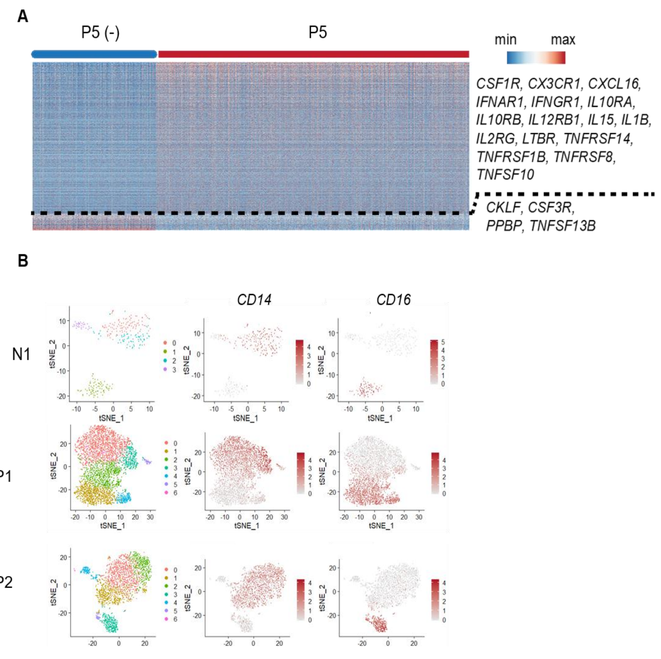
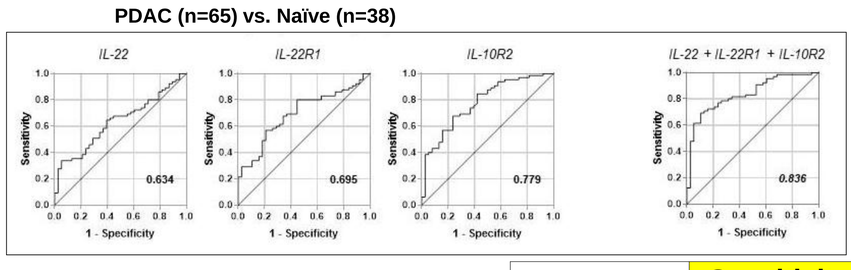


Figure 4. Identification of new PDAC specific targets from scRNA-seq. Given the strong expansion of the monocyte/macrophage compartment in IL-10R2+ PBMCs of patients with PDAC, we next sought to dissect the subpopulation structure of this compartment. We analyzed the expression of CD14 and CD16, two surface markers classifying monocytes into classical (CD14+CD16-), intermediate (CD14+CD16+), and nonclassical (CD14-CD16+) types. The IL-10R2+ and IL-10R2- monocyte/macrophage subsets were distinguished by the expression of CD16, indicating the enrichment of nonclassical monocytes in IL-10R2+ cells. The monocyte/macrophage subset in IL-10R2+ cells exhibited high levels of *NEURL1, ID2, TMPO*, and *DUT*, which belong to a signature gene set associated with recently characterized tumor-educated monocytes (TEM)

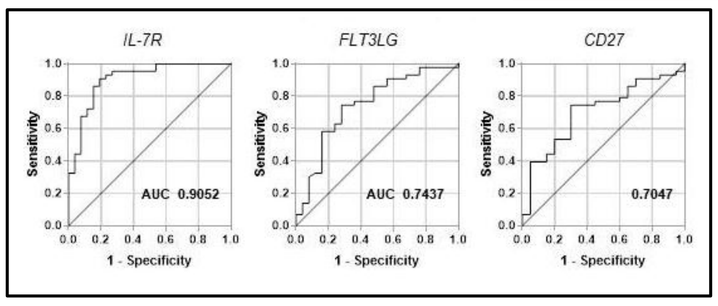
Figure 5. Determination of the clinical usefulness of IL-10R2-positive cells for PDAC diagnosis and monitoring. IL-22, IL-22R1, and IL-10R2 gene expression analyses by qPCR using PBMC from naïve, pancreatitis, biliary cancer, and PDAC groups. (A) ROC curve for IL-22, IL-22R1, and IL-10R2 in PBMCs from PDAC versus Naive. (B) ROC curve for IL-7R, CD27, and FLT3L three markers found from scRNA-seq experiment.

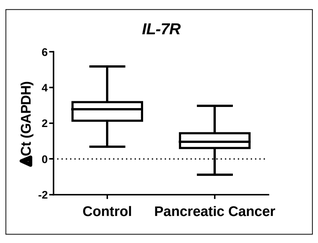
PDAC (n=65) vs. Naïve (n=38)



	Sensitivity	Specificity	p-value
IL-10R2	0.677	0.763	<.0001
IL-22R1	0.569	0.789	0.001
IL-22	0.338	0.947	0.032

	Sensitivity	Specificity	p-value
IL-7R	0.861	0.845	<.0001
FLT3LG	0.744	0.720	0.009
CD27	0.743	0.701	0.009

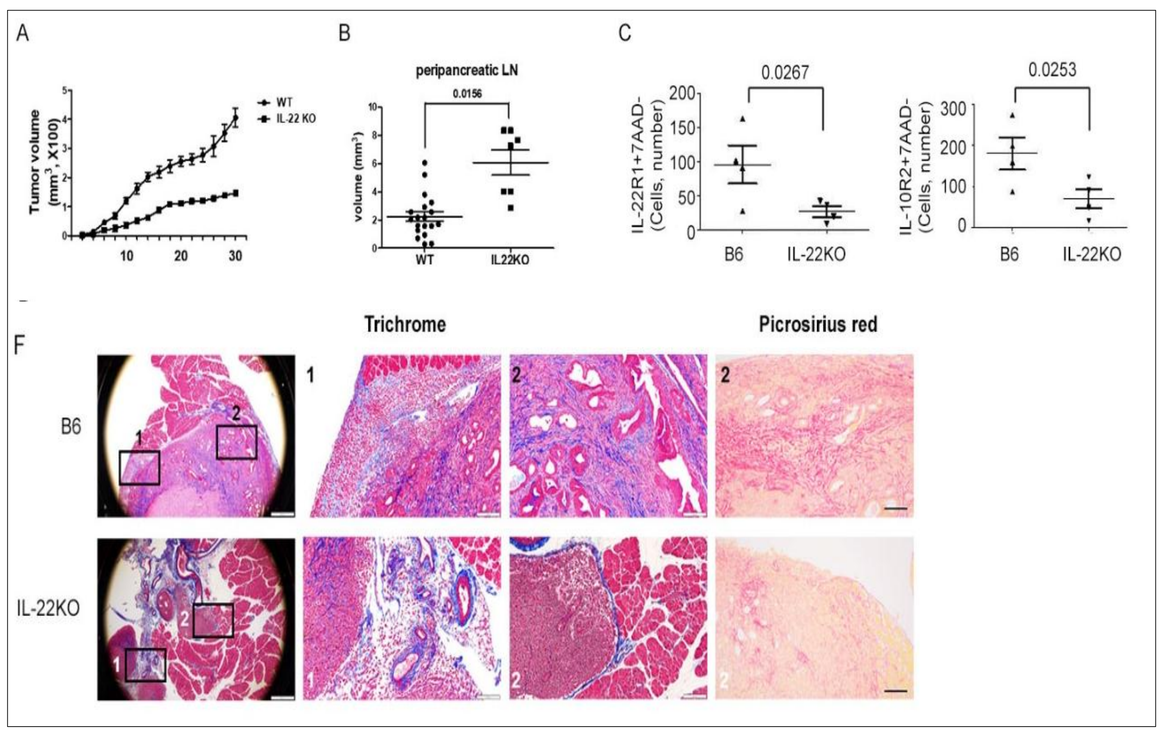




Area under the ROC curve	*IL-7R*
Area	0.9052
Std. Error	0.03884
95% confidence interval	0.8291 to 0.9813
P value	<0.0001

ΔCT Cutoff	Sensitivity	95% CI	Specificity	95% CI	Likelihood ratio
< 1.633	**86.05**	**72.74% to 93.44%**	**84.62**	**66.47% to 93.85%**	**5.593**
< 1.696	86.05	72.74% to 93.44%	80.77	62.12% to 91.49%	4.474
< 1.789	88.37	75.52% to 94.93%	80.77	62.12% to 91.49%	4.595
< 1.949	**90.70**	78.40% to 96.32%	**80.77**	62.12% to 91.49%	4.716

Figure 6. Reduction of tumor volumes and IL-10R2+ myeloid cell infiltration in the IL-22 KO mouse model. Orthotopic syngeneic PDAC mouse models were established by injecting Pan02 cells (2 × 106 cells/mouse) into the pancreas of WT and IL-22 KO C57BL/6 mice. (A) Tumor volume was calculated at serial intervals by measuring the long (D) and short (d) diameter of the tumor and applying the formula V = 1/2(D × d2). (B) Volume of peripancreatic lymph nodes. Seven mice in each group from WT and IL-22 KO mice were evaluated. (C) Quantification of IL-10R2+ cells and IL-22R1+ cells in whole blood cells by flow cytometry (Wilcoxon rank sum test). (F) Masson's trichrome and Picrosirius red staining of tumor tissue sections of the pancreas from WT and IL-22 KO mice. Scale bar, 500 μm (upper), 100 μm (lower).



4. Conclusion

IL-10R2/IL-22R1 expressed PCMIC were specifically expressed highly in PDAC patients, correlated with the mass size, and alarmed the cancer recurrence after surgery. From the scRNA-Seq from the PDAC specific IL-10R2+ cell, we successfully found three markers which made more precisely delineate PDAC from other diseases as well as healthy controls.