췌장 이미징 윈도우을 이용한 췌관 선암종 종양 미세환경의 장기적 세포수준 시각화

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Longitudinal visualization of pancreatic ductal adenocarcinoma tumor microenvironment using pancreas imaging window

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancer with a fibrotic microenvironment that acts as a barrier decreasing the therapeutic efficacy of the most anti-cancer drug. Immune checkpoint inhibitors (ICIs) treatment has also shown disappointing results in treating the PDAC. An in-depth study of the PDAC tumor microenvironment using the proper preclinical model is necessary to uncover the underlying mechanism of immune evasion of PDAC. This study focused on establishing a method for the longitudinal visualization of the pancreas in the orthotopic PDAC mouse model using a pancreas imaging window. We successfully visualized the tumor growth, morphological and molecular changes of blood vessels repetitively at the same site in a live Panc02 PDAC mouse model for 10 days.

1. 연구 배경

Pancreatic ductal adenocarcinoma (PDAC), a malignant epithelial neoplasm that arises from the pancreatic duct cells, accounts for 85-95 percent of all solid pancreatic tumors [1]. PDAC is a highly lethal disease and resistant to the most of existing therapies, resulting in a 5-year overall survival rate of less than 10%. Many factors contribute to the poor prognosis of PDAC including desmoplastic reaction, which leads to the excessive extracellular matrix deposition creating a physical barrier and reducing the effective drug delivery to the tumor. These features give rise to the immunosuppressive tumor microenvironment; even the immune checkpoint inhibitors (ICIs) treatment that has successfully treated several types of cancer have shown disappointing results in treating the PDAC [2]. Although the underlying mechanism remains unclear, one potential cause is the insufficient tumor-reactive CD8+ T cells infiltration which is essential for antitumor immunity for cancer immunotherapies with ICIs. However, a more in-depth study of the PDAC tumor microenvironment is necessary to uncover the complex underlying mechanism of the low efficacy of immunotherapies with ICIs.

In recent years, there have been notable improvements in understanding the molecular mechanism that governs the development of PDAC and increasing numbers of new immunotherapy is entering clinical treatments. Many recent studies have employed PDAC mouse models, especially the orthotopic syngeneic mouse model that allows the injection of PDAC cells into the pancreas of the immunocompetent mouse. It can be performed in large cohorts of mice, thus providing a cost-effective and repeatable model for evaluating the therapeutic agents. Moreover, the orthotopic mouse model offers a similar microenvironment for tumor development, making it more clinically relevant than the subcutaneous or chemically-induced models. However, until now, the preclinical studies of PDAC were mainly performed by *ex vivo* observation, which provides relatively limited knowledge of spatiotemporal dynamic changes at cellular and molecular levels over time. Direct *in vivo* dynamic observation is imperative to elucidate a thorough investigation of the immunotherapy treatment of PDAC. Moreover, continuous observation of the dynamically changing PDAC tumor microenvironment will greatly improve the efficiency and accuracy in assessing the treatment response by novel therapeutics.

A previous study reported a novel pancreas imaging window for stable and repetitive imaging on the same site in the pancreas over several weeks [3]. In this study we focused on establishing a pancreas imaging window technique for a longitudinal visualization of the pancreas in the orthotopic Panc02 mouse model of PDAC. We successfully and longitudinally visualized the PDAC growth, desmoplastic reaction, vascular remodeling, and HEVs development at the same sites in the pancreas of the Panc02 PDAC mouse model for 10 days.

2.연구 방법

For the orthotopic mouse model, we followed the surgical technique developed before[4]. This study used 2 million Panc02-GFP cells (pancreatic tumor cells that express green fluorescence protein). The 2 million Panc02 cells in 10 μl Matrigel were injected into the pancreas of the C57BL/6J mouse. For longitudinal observation of the pancreatic tumor, the orthotopic modeling methods were combined with the pancreas window implantation (Fig. 1). To implant the pancreas window (Fig. 2a), first, the spleen and pancreas were overlaid on the window plate. The window was then secured by suturing the purse string encircling the window. After adjusting the pancreas and spleen again, the 2 million Panc02 cells were injected before the 12mm cover glass was employed to cover the top of the window.

To visualize the blood vessels and high endothelial venules, 25 μg anti-CD31 conjugated with Alexa Fluor 555 and 25 μg anti-MECA79 conjugated with Alexa Fluor 647 were injected intravenously injection 1-3 hours before imaging. For the

stability of the imaging process, the pancreatic window was attached to the customized window holder (Fig. 2b) that matched the size of the pancreatic window. During the imaging, mouse temperature was also maintained at 37°C with a heating

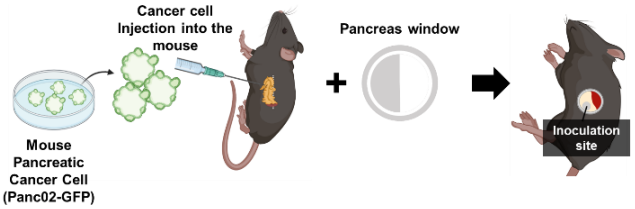

Figure 4. Experimental methods of the integration between the orthotopic modeling and pancreas window implantation

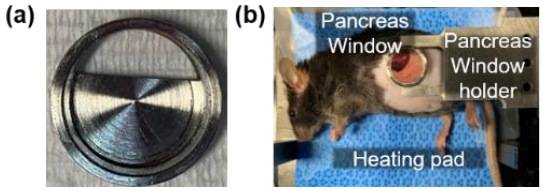

Figure 4. (a) pancreas window. (b) photo of mouse with the pancreas window prepared for intravital imaging

pad under the mouse.

3. 연구 결과

The pancreas imaging window was first established to separate the soft-sheet-like pancreas tissue from the nearby bowel movement and keep the pancreas position in the same place, thus allowing us to observe the specific region of the pancreas continuously [3]. In this study, we combined the pancreas window and the orthotopic pancreas tumor modeling to observe more thoroughly the tumor growth on the inoculation site of the tumor cells (Fig. 3).

By utilizing the pancreas imaging window, we successfully observed the Panc02 tumor growth from the day of the inoculation for up to 15 days (Fig. 3). In addition, herein, we longitudinally performed *in vivo* visualization of the PDAC to visualize the tumor growth and the tumor-associated blood vessel alteration. Interestingly, we found the formation of the MECA79 labeled PNAd expressing high endothelial venules (HEVs) in the peri-tumor region. HEVs, specialized blood vessels characterized by plump endothelial cells, have a role in facilitating lymphocyte trafficking in the secondary lymphoid organs (SLOs). Notably, HEVs have been reported to develop in non-lymphoid organs during inflammation, including tumor tissue. Tumor-associated HEVs (TA-HEVs) could facilitate the recruitment of the lymphocytes into the tumor site, which was observed in several cancer types and associated with a good prognosis.

The investigation of the treatment effect in the preclinical model has been mainly conducted by *ex vivo* observation of the resected tissue, which provides relatively limited knowledge of spatiotemporal dynamics at cellular and molecular levels. Here, our work is highlighted to directly visualize the *in vivo* pancreas tumor microenvironment over extended period of time. Our study demonstrated the longitudinal observation of the pancreas tumor microenvironment for up to 10 days, especially the modification of the tumor vessels, the formation of HEVs, and the tumor cells growth. This intravital longitudinal imaging method could serve as a useful method for further observation of the HEV's role in the tumor

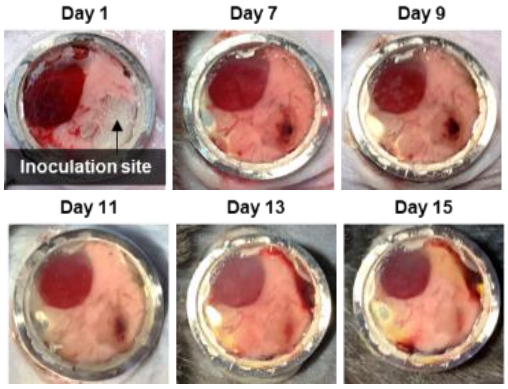

Figure 1. Pancreas window with the pancreas that has been injected with tumor cells and the tumor growth from day 1 until day 15

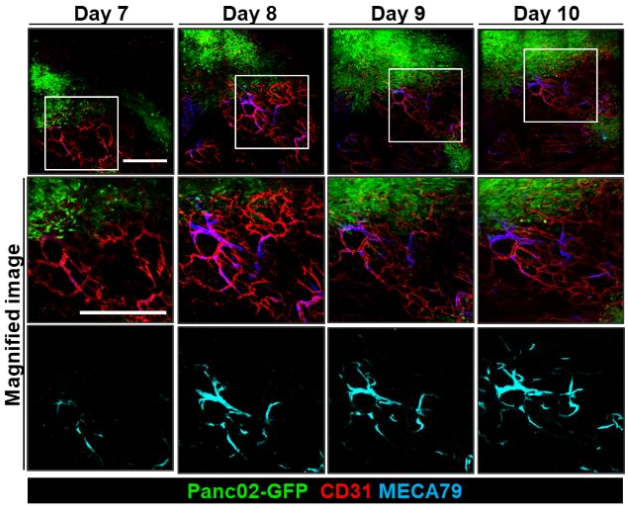

Figure 2. (top) tumor microenvironment condition up to 10 days (Green: Panc02-GFP cells, Red: blood vessel (CD31), Blue: HEVs (MECA79)), (middle) magnified images of the top figures. (bottom) HEVs development. Scale bar: 500μm

microenvironment and studying the effect of immunotherapy treatment at a cellular level.

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