Assays for orthotopic PDAC mouse models

Do Young Hyeon  
Seoul National University  https://orcid.org/0000-0002-1825-0097

Dowoon Nam  
Korea University

Youngmin Han  
Seoul National University College of Medicine

Duk Ki Kim  
Seoul National University College of Medicine

Gibeom Kim  
Seoul National University

Daeun Kim  
Ajou University

Jingi Bae  
Korea University

Seunghoon Back  
Korea University

Dong-Gi Mun  
Korea University

Inamul Hasan Madar  
Korea University

Hangyeore Lee  
Korea University

Su-Jin Kim  
Korea University

Hokeun Kim  
Korea University

Sangyeop Hyun  
Ajou University

Chang Rok Kim  
Seoul National University

Seon Ah Choi  
Seoul National University

Yong Ryoul Kim  
Seoul National University
Juhee Jeong  
Seoul National University College of Medicine

Suwan Jeon  
Seoul National University College of Medicine

Yeon Woong Choo  
Seoul National University College of Medicine

Kyung Bun Lee  
Seoul National University College of Medicine

Wooil Kwon  
Seoul National University College of Medicine

Seunghyuk Choi  
Hanyang University

Taewan Goo  
Seoul National University

Taesung Park  
Seoul National University

Young-Ah Suh  
Seoul National University College of Medicine

Hongbeom Kim  
Seoul National University College of Medicine

Ja-Lok Ku  
Seoul National University College of Medicine

Min-Sik Kim  
Daegu Gyeongbuk Institute of Science and Technology

Eunok Paek  
Hanyang University

Daechan Park (dpark@ajou.ac.kr)  
Ajou University

Keehoon Jung (keehoon.jung@snu.ac.kr)  
Seoul National University College of Medicine

Sung Hee Baek (sbaek@snu.ac.kr)  
Seoul National University

Jin-Young Jang (jangjy4@snu.ac.kr)  
Seoul National University College of Medicine

Dahee Hwang (dahee@snu.ac.kr)  
Seoul National University

Sang-Won Lee (sw.lee@korea.ac.kr)  
Korea University
Abstract

The associated publication reports proteogenomic analysis of human pancreatic ductal adenocarcinoma (PDAC), where we provided significantly mutated genes (SMGs)/biomarkers, cellular pathways, and cell types as potential therapeutic targets to improve stratification of patients with PDAC. This protocol describes the detailed methods for assays for orthotopic PDAC mouse models, including mouse tumour tissue processing, ultrasound imaging, Masson-trichrome staining, and IHC analysis for immune cell markers.

Procedure

Mouse tumour tissue processing

Briefly, the tumour tissues were resected, chopped into small pieces, and digested in a 5% CO₂ incubator at 37 °C for 20 min with RPMI-1640 (Biowest, L0498) containing collagenase type IV (Worthington Biochemical, LS004189, 1 mg/mL), hyaluronidase (Sigma, H6254, 1 mg/mL), and DNase I (Sigma, DN25, 1.5 mg/mL). The digested tissues were minced, filtered through a 70 μm cell strainer, and made into single-cell suspensions.

Ultrasound imaging

Ultrasound imaging was performed using a Vevo 2100 System (VisualSonic) with an LZ550 probe (frequency 40 MHz) to measure the size of each tumour twice a week, 7 days after the injection of the cancer cells until the end of the study. Tumour-bearing mice were anaesthetised with an intraperitoneal injection of a mixed solution of ketamine (100 mg/kg) and xylazine (10 mg/kg). The skin was covered with ultrasound gel, and images were acquired using a probe. Tumour tissues in the abdominal cavity were defined as a different echogenicity from the normal pancreas on ultrasound imaging. The long diameter (LD) and short diameter (SD) were measured. Tumour volume was calculated using the following formula: tumour volume = (LD × SD²)/2.

Masson-trichrome staining

For direct visualization of collagen deposition, Masson-trichrome staining was performed. Briefly, paraffin-embedded, formalin-fixed tissue blocks were cut into 4-μm thick slices. The slices were deparaffinized and then subjected to Masson's trichrome staining by using reagents and kits from Sigma-Aldrich following the manufacturer's protocols. Slides were analyzed using Nikon Eclipse Ts2R microscope and the image quantification was performed by using Image J program.
**IHC analysis**

Paraffin-embedded, formalin-fixed tissue blocks were cut into 4 μm thick slices. IHC analysis was performed for CD66b, CD3, CD4, and CD8 using the Ventana BenchMark XT Staining system. Briefly, the IHC sections were baked and deparaffinised, and antigen retrieval was performed for 24 min at 100 °C with Cc1 solution (Ventana, #05279801001). Endogenous peroxidase was blocked using 3% hydrogen peroxide for 4 min at 37 °C. The following primary antibodies were then used: anti-CEACAM8/CD66B (G10F5 clone, Novus Biologicals, Cat: NB100-77808, 1:100 dilution) as a PMN-MDSC marker; anti-CD3 (polyclonal, DAKO, Cat: A0452, pre-diluted) as a pan-T cell marker, anti-CD4 (SP35 clone, Ventana, Cat: 790-4423, pre-diluted) for helper T cells, and anti-CD8 (SP57 clone, Ventana, Cat: 790-4460, pre-diluted) for cytotoxic T cells. An OptiView universal DAB kit (Ventana, #760-700) was used for the enzyme-substrate reaction. After immunostaining, the slides were counterstained with haematoxylin, and the haematoxylin shading was changed to a blue colour by applying a bluing agent. IHC assessments were performed using a Nikon Eclipse Ts2R microscope (Nikon, Japan). For each marker, the cells were manually counted in five randomly chosen areas per tissue section using sections from 10 patients.