Abstract. Background: To effectively use pancreatic cancer patient-derived xenograft (PDX) models in translational research, successful PDX engraftment of surgical specimens in immune-deficient mice is needed. Materials and Methods: A total of 102 patients underwent pancreatic cancer resection using various procedures. Tumor tissue from all patients was implanted subcutaneously into mice. Tumor engraftment and growth in mice were determined. Engraftment was tested for correlation with operation type, time needed to remove the specimen, tumor differentiation, lymph node metastasis, and protein expression of p53, Receptor tyrosine-protein kinase erbB-2 (CERBB2), or deleted in pancreatic carcinoma locus 4 (DPC4). Results: Multivariate analysis showed that a tumor size of more than 3.5 cm in the patient was a significant factor related to successful PDX engraftment. In contrast, there was no correlation of engraftment with surgical procedure, time needed to remove the specimen, tumor differentiation, lymph node metastasis, and protein expression of p53, Receptor tyrosine-protein kinase erbB-2 (CERBB2), or deleted in pancreatic carcinoma locus 4 (DPC4). Conclusion: A minimum tumor size in the patient is an important factor for successful tumor engraftment.

In 1992, Fu et al. constructed an orthotopic metastatic mouse model of human pancreatic cancer with histologically intact patient specimens (1). In 2014, Walters et al. reported the clinical, molecular, and genetic correlation between a model of human pancreatic cancer using a patient-derived xenograft (PDX) and the original human tissue (2). Kim et al. reported the molecular similarities between patient specimens after neoadjuvant chemotherapy and in the subsequent PDX model (3). However, the success rate of tumor engraftment of pancreatic cancer in immunodeficient mice is variable (4, 5). To effectively use PDX models in translational research (6-8), it is important to analyze factors that correlate with their successful engraftment (4, 9). In this study, we analyzed the surgical and oncological factors affecting PDX generation of pancreatic ductal adenocarcinoma (PDAC).

Materials and Methods

Patients and tumor acquisition. Between October 2013 and June 2015, a total of 115 consecutive patients with pancreatic cancer underwent surgical resection with curative intent at the Asan Medical Center (Seoul, South Korea). The surgical procedure was determined by the location of the tumor. A retrospective medical
record review was performed. Of the 115 patients, those who did not have PDAC were excluded. The remaining 102 patients were analyzed in detail according to surgery- and oncology-related factors. Surgical procedures were dependent on tumor site or laparotomy method, the time needed to remove the specimen, and the status of vessel resection. Either pylorus-preserving or classic pancreatico-duodenectomy (PD) was performed to remove tumors of the head or uncinate of the pancreas. Distal pancreatectomy with splenectomy (DP) was performed on lesions in the pancreatic body or neck. The operations included both open and laparoscopic procedures. Oncology-related factors were T-stage, tumor differentiation, lymph node (LN) metastasis, and genetic alteration of p53, Receptor tyrosine-protein kinase erbB-2 (CERBB2), or deleted in pancreatic carcinoma locus 4 (DPC4). Following surgical resection, pathological examinations were performed to determine tumor characterization. The remaining tumor tissues were collected in serum-free RPMI media at 4°C for xenografting. This study was performed with approval of the Institutional Review Board (IRB) at the Asan Medical Center (IRB No. 2013-0939).

Tumor implantation into mice. The animal care protocol for this study was approved by the Animal Care and Use Committee of the University of Ulsan. Eight-week-old male, nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice were used for tumor engraftment. They were kept in positive pressure rooms with filtered and humidified air under specific pathogen-free conditions. All of the animals were anesthetized with intraperitoneal injection of xylazine (10 mg/kg) and ketamine (80 mg/kg) during tumor implantation. 102 tumor tissues of human PDAC were obtained from pancreatic surgery. The tumors were mechanically minced into small fragments (1-2 mm³) and implanted subcutaneously into the flank of each mouse. To verify the tumor establishment of the tumor model, the tumors were monitored for at least 100 days and measured until a volume of 1000 mm³ was reached.

Immunohistochemistry. Tumors were fixed in 10% formalin for 24 h and embedded in paraffin. Both human and mouse tumor tissues were sectioned at a 4 μm thickness and stained with hematoxylin and eosin (H&E). A board-certified pathologist specializing in pancreatic cancer reviewed the slides to compare the tumor architecture and desmoplastic appearance. Immunohistochemistry (IHC) was performed to quantify the expression of p53, C-ERBB2, and DPC4 in the primary human tumors, as previously described (10). Briefly, after deparaffinization and antigenic retrieval, the slides were labeled with a monoclonal antibody against p53 (clone DO-7, 1:3000; DAKO, Glostrup, Denmark) and a polyclonal antibody against C-ERBB2 (clone 4B5, 1:500; Ventana, USA) or DPC4 (clone EP618Y, 1:100; GeneTex, Irvine, CA, USA). Labeling was detected using the avidin-biotin complex staining method. Either 3,3′-diaminobenzidine (DAB) (for p53 and C-ERBB2) or 3-amino-9-ethylcarbazole (for DPC4) was used as the chromogen. Normal saline was used as a substitute for the primary antibody in the negative control reaction.

Statistical analysis. All of the statistical analyses were conducted using SPSS for Windows, version 21.0 (IBM Corp Armonk, NY,
USA). Descriptive statistics for categorical variables are presented as relative frequencies. The Chi-square test or Fisher’s exact test was applied depending on the number of cases in each subgroup. A logistic regression model was performed to verify significant factors for successful PDX generation in multivariate analyses. \( p \)-Values less than 0.05 were considered statistically significant.

Results

Clinicopathological characteristics of the enrolled patients. We obtained fresh pancreatic tumor tissue from 115 patients for the PDX model. Among these case, 13 patients were diagnosed with non-ductal adenocarcinoma and were excluded. The remaining 102 patients were finally enrolled for xenograft study (Figure 1). The demographics, surgical, and oncological features of these patients are listed in Table I. The average age of patients was 61.9 years, and males comprised 54.9% of the study population. PD (n=67, 65.7%) was performed more frequently than DP (n=35, 34.3%) and the time to specimen removal in PD and DP was 187.4±52.1 min and 159.2±45.2 min, respectively. Approximately 80% of surgeries were performed with open method (n=81, 79.4%). The average size of the human tumor was 3.5 (±1.1) cm and all cases were T3 stage. Lymph node (LN) metastasis was presented in 65.7% of the patients. Finally, expression of p53 was altered in 18.6% of cases, C-ERBB2 in 14.7%, and DPC4 in 54.9%.

PDX generation in NOD/SCID mice. After transplantation, we confirmed that the tumors from 57 patients were successfully grafted in NOD/SCID mice. The average time to tumor formation at the subcutaneous site was 57±2 days, and the tumor volume rapidly increased (Figure 2 A and B). To observe the tumor architecture and desmoplastic appearance, H&E staining was performed for the original human primary tumor and the subsequent PDX tumor (Figure 2C). Tumor differentiation, atypical glandular structure, and stromal content were preserved during PDX formation.

Surgical and oncological factor analysis. To identify significant factors affecting PDX generation, surgery- and oncology-related factors were analyzed. Univariate analysis showed that tumor size was the only significant factor that correlated with successful PDX generation (Table II). The type of operation, time needed to remove the specimen, status of vessel resection, tumor differentiation, metastatic LN status, and alterations of p53, C-ERBB2 and DPC4 were not associated with PDX generation. Multivariate analysis demonstrated that a tumor size greater than 3.5 cm was independently associated with successful PDX generation (odds ratio: 4.715, 95% confidence interval: 1.827-12.171; \( p=0.001 \)).

Discussion

A common barrier to preclinical and clinical studies of pancreatic cancer is the difficulty in obtaining a sufficient amount of tumor tissue, which is important for a detailed analysis and understanding of the molecular characteristics of this disease (11, 12).

Recently, several studies using pancreatic PDX models have been published. Mattie et al. reported that gene-expression patterns of primary tumors were maintained in PDX models after extensive passaging (13). Garrido-Laguna et al. reported that PDX models were generated in 61% of patients, and that successful engraftment predicted poor patient survival (5). In our experience, the rate of PDX generation was 55.8% and it took approximately 2 months to confirm the establishment of tumor in SCID mice.

To effectively use PDX models in translational research, it is necessary to increase the tumor engraftment rate. Garrido-Laguna et al. reported that rate of PDX generation for PDAC correlated with DPC4 inactivation (5). However, there have been few reports on the factors related to successful PDX generation. We speculated that surgery-related factors, such as
tissue status after surgical resection, as well as malignancy of the tumor, may influence PDX generation. Therefore, we analyzed surgical factors including PD versus DP, open versus laparoscopy, status of vessel resection, and the time needed to remove the specimen, and did not find any correlation of these factors with PDX generation. The oncology-related factors evaluated included tumor size, extension and differentiation, the status of LN metastasis, and alterations of p53, C-ERBB2 and DPC4. Among these factors, only tumor size greater than 3.5 cm was related to successful PDX generation. This may be due to the fact that larger tumors are more aggressive and have higher metabolic activity. Several articles have reported that a tumor size above 3 cm is an independent prognostic factor in PDAC (14, 15). In addition, larger lung tumors have been shown to correlate with higher heterogeneity and metabolic activity (16, 17).

In summary, we analyzed surgical and oncological factors in patients with PDAC to identify those that may affect PDX generation, and found that the size of the primary tumor is a significant factor. Additional molecular studies are needed to fully understand how tumor size results in better engraftment success rates for pancreatic PDX models. It is also essential that future studies use orthotopic implantation in mice to generate patient-derived orthotopic xenograft models, since they better represent the tumor behavior, such as metastasis, in the patient (18-20).

Financial Support and Competing Interests

The Authors declare that they have no competing interests.

Acknowledgements

This study was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea. (HI14C2640) and a grant of the Asan Institute for Life Sciences, Seoul, Korea (2015-7213).

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Received November 18, 2015
Revised December 21, 2015
Accepted December 23, 2015