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BASIC AND TRANSLATIONAL—PANCREAS

Administration of Gemcitabine After Pancreatic Tumor Resection in Mice Induces an Antitumor Immune Response Mediated by Natural Killer Cells

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BACKGROUND & AIMS: Even after potentially curative R0 resection, patients with pancreatic ductal adenocarcinoma (PDAC) have a poor prognosis owing to high rates of local recurrence and metastasis to distant organs. However, we have no suitable transgenic animal models for surgical interventions. **METHODS:** To induce formation of pancreatic tumor foci, we electroporated oncogenic plasmids into pancreata of LSL-KrasG12D × p53fl/fl mice; mutant Kras was expressed in p53fl/fl mice using a sleeping beauty transposon. We co-delivered a transposon encoding a constitutively active form of Akt2 (myrAkt2). Carcinogenesis and histopathologic features of tumors were examined. Metastasis was monitored by bioluminescence imaging. Tumors were resected and mice were given gemcitabine, and tumor recurrence patterns and survival were determined. Immune cells were collected from resection sites and analyzed by flow cytometry and in depletion experiments. **RESULTS:** After electroporation of oncogenic plasmids, mice developed a single pancreatic tumor nodule with histopathologic features of human PDAC. Pancreatic tumors that expressed myrAkt2 infiltrated the surrounding pancreatic tissue and neurons and became widely metastatic, reflecting the aggressive clinical features of PDAC in patients. Despite early tumor resection, mice died from locally recurring and distant tumors, but adjuvant administration of gemcitabine after tumor resection prolonged survival. In mice given adjuvant gemcitabine or vehicle, gemcitabine significantly inhibited local recurrence of tumors, but not metastasis to distant organs, similar to observations in clinical trials. Gemcitabine inhibited accumulation of CD11b+Gr1intF4/80int myeloid-derived suppressor cells at the resection margin and increased the number of natural killer (NK) cells at this location. NK cells but not T cells were required for gemcitabine-mediated antitumor responses. **CONCLUSIONS:** Gemcitabine administration after resection of pancreatic tumors in mice activates NK cell–mediated antitumor responses and inhibits local recurrence of tumors, consistent with observations from patients with PDAC. Transgenic mice with resectable pancreatic tumors might be promising tools to study adjuvant therapy strategies for patients.

Keywords: Pancreatic Cancer; Surgery; Adjuvant Therapy; Resectable Transgenic Mice.

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related death with a 5-year survival less than 5%.[1–4] Aggressive local tissue infiltrations including neural invasions and early metastasis are typical clinical features of PDAC. Surgical resection is the only curative option for nondisseminated PDAC. However, even after early complete resection the prognosis remains poor owing to recurrence rates of 80%–85%. To improve disease-free and overall survival, adjuvant chemotherapy strongly is recommended.[5–7] Despite advances in adjuvant chemotherapy, most PDAC patients die within 2 years after surgery. In a large phase III study (CONKO-001), adjuvant gemcitabine achieved a 20.7% 5-year overall survival rate compared with 10.4% in the observation group and a prolonged median survival of 22.1 vs 20.2 months.[8] The development of new therapeutic strategies is needed urgently, but rapid advancements appear unlikely owing to

***Authors share co-first authorship.**

Abbreviations used in this paper: **ADM, acinar-to-ductal metaplasia; CONKO-001, Charité Onkologie-001; EGFP, enhanced green fluorescent protein; EP, electroporation technique; Erk, extracellular signal–regulated kinase; KPfl, LSL-Kras-G12D × p53fl/fl; MDSC, myeloid-derived suppressor cell; NK, natural killer; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; Pfl, Kras-G12V–expressing transposon in p53fl/fl.**

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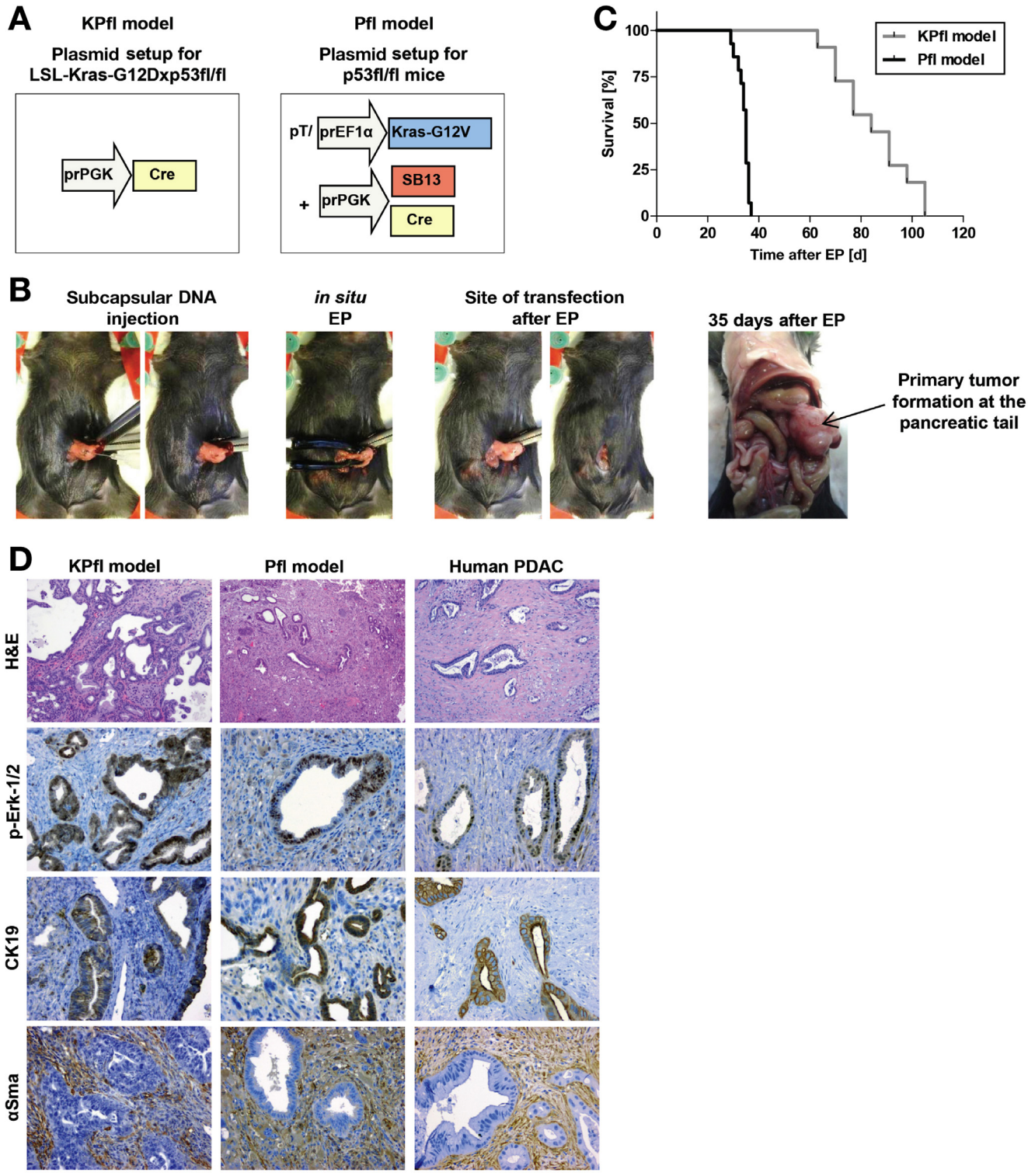

Figure 1. In situ electroporation of the pancreatic parenchyma promotes the development of a focal primary tumor through local induction of oncogenic Kras and p53 deletion. (*A*) Two alternative plasmid set-ups for local injection and electroporation into the pancreatic tail to induce a single tumor. *Left*: A Cre recombinase expressing plasmid was used in LSL-Kras-G12D × p53fl/fl mice (KPfl model) to initiate endogenous expression of the Kras-G12V oncogene. *Right*: Plasmids containing a Kras-G12V encoding transposon, the sleeping beauty transposase SB13, and the Cre recombinase were used in p53fl/fl mice, reflecting a p53-deleted model with overexpression of oncogenic Kras (Pfl model). (*B*) Images showing intrapancreatic DNA injection, subsequent transfection by local electroporation, and local growth of a single tumor nodule at the transfection site (Pfl model, 35 days after EP). (*C*) Kaplan–Meier survival curve showing the KPfl (n = 12) and Pfl (n = 14) models. (*D*) Tumors of KPfl and Pfl models were harvested and histologically examined. Human PDAC specimens were stained for comparison. The figures show H&E (original magnification, 100×), phospho-Erk-1/2, cytokeratin 19 (CK19), and α-smooth muscle actin (α-SMA) (original magnification, 200×) stainings of tumor tissues.