

A Syngeneic Orthotopic Murine Model of Pancreatic Adenocarcinoma in the C57/BL6 Mouse Using the Panc02 and 6606PDA Cell Lines

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Key Words

Immunocompetent mice · Mouse cell lines · MRI · Orthotopic murine model · Panc02 · Pancreatic cancer · 6606PDA

Abstract

Background/Aims: To develop a clinically relevant immunocompetent murine model to study pancreatic cancer using two different syngeneic pancreatic cancer cell lines and to assess MRI for its applicability in this model. **Methods:** Two cell lines, 6606PDA and Panc02, were employed for the experiments. Cell proliferation and migration were monitored in vitro. Matrigel™ was tested for its role in tumor induction. Tumor cell growth was assessed after orthotopic injection of tumor cells into the pancreatic head of C57/BL6 mice by MRI and histology. **Results:** Proliferation and migration of Panc02 were significantly faster than those of 6606PDA. Matrigel did not affect tumor growth/migration but prevented tumor cell spread after injection thus avoiding undesired peritoneal tumor growth. MRI could reliably monitor longitudinal tumor growth in both cell lines: Panc02 had a more irregular finger-like growth, and 6606PDA grew

more spherically. Both tumors showed local invasiveness. Histologically, Panc02 showed a sarcoma-like undifferentiated growth pattern, whereas 6606PDA displayed a moderately differentiated glandular tumor growth. Panc02 mice had a significantly shorter (28 days) survival than 6606PDA mice (50 days). **Conclusion:** This model closely mimics human pancreatic cancer. MRI was invaluable for longitudinal monitoring of tumor growth thus reducing the number of mice required. Employing two different cell lines, this model can be used for various treatment and imaging studies.

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Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the Western hemisphere. Its annual incidence is steadily rising and it is responsible for approximately 33,000 cancer deaths in the United States and

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almost 13,000 in Germany [1–3]. The late onset of symptoms, early development of metastases and the high morbidity associated with surgery add to its often rapid and fatal course. This is mirrored by diagnoses usually made in late stages of disease as well as the general lack of effective adjuvant and palliative treatment options [4].

At the cellular as well as the molecular level, several mechanisms have been described that enable the cancer cell to evade the surveillance of the immune system. These include the loss of co-stimulatory molecules such as ICAM-1 or B-7 [5–7] or the loss of functional death receptors like Fas [8, 9]. Pancreatic cancer cells may also actively suppress the immune response by factors like IL-10, glycoproteins or FasL [8–12]. Furthermore, IL-10 and TGF- β are elevated in sera of pancreatic cancer patients and could induce a general state of immunosuppression. This may promote tumor growth and the formation of disseminated disease as evidenced by disseminated tumor cells in >40% of cancer patients [13].

Despite the advances in cancer immunology and improvement in surgical as well as non-surgical therapeutic approaches in patients with metastases [14], the overall 5-year survival rate of 5% has not changed significantly [3]. Hence, models that resemble the human situation in pancreatic cancer are required to study the underlying causes of immunosuppression. Also, these models can be helpful in developing new preclinical treatment modalities hopefully leading to effective therapies against pancreatic cancer. Here, we introduce a syngeneic orthotopic murine model using two different murine pancreatic adenocarcinoma cell lines: the well-differentiated, slowly growing modestly aggressive cell line 6606PDA and the low/undifferentiated fast growing highly aggressive cell line Panc02. For longitudinal studies of tumor growth, a 7-tesla MRI scan was employed.

Materials and Methods

Laboratory Animals

Eight-week-old male C57/BL6 mice with a body weight of 20–23 g were obtained from the Charles River Laboratories (Bad Sulzfeld, Germany) and allowed to adapt to the new surrounding for 14 days. They were maintained in a specific pathogen-free environment receiving sterilized food (ssniV R-Z, ssniV Spezialdiäten GmbH, Soest, Germany) and tap water ad libitum. Stress levels were kept to a minimum before being used in experiments. Animal rooms had a 12-hour light/dark cycle and were maintained at constant temperature and humidity. All animal studies had been approved by the Ethics Committee for Animal Care of Mecklenburg-Vorpommern, Germany.



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Fig. 1. Technique of tumor cell injection into the head of the pancreas using Matrigel.

Cell Lines and Culture

The murine pancreatic adenocarcinoma cell line 6606PDA, containing a K-ras G12D mutation, was a kind gift from Prof. Tuvesson, Cambridge, UK. The murine pancreatic adenocarcinoma cell line Panc02 was developed by Corbett et al. [15]. 6606PDA was maintained in RPMI-1640 medium and Panc02 in DMEM. Both media were supplemented with 10% fetal calf serum, 100 U/ml of penicillin and 100 μ g/ml of streptomycin (referred to as ‘complete medium’). Tissue culture reagents were obtained from Gibco (Invitrogen, Carlsbad, Calif., USA). Cell cultures were kept in a humidified incubator at 37°C with 5% CO₂.

Orthotopic Injection of Tumor Cells

Subconfluent cultures of tumor cells were harvested using a 0.05% trypsin solution, washed twice in PBS and resuspended as single-cell suspensions in serum-free RPMI-1640 or DMEM. Cell viability was tested using trypan blue exclusion. Viability was always >95%.

General anesthesia in mice was induced using a combination of ketamine hydrochloride (Ketanest S®; Pfizer Pharma, Berlin, Germany) and xylometazoline hydrochloride (Rompun®; Bayer Healthcare, Berlin, Germany) at concentrations of 87 and 13 mg/kg i.p. The abdominal cavity was opened by a 1.5-cm-wide transverse laparotomy pointing slightly to the right. The head of the pancreas was identified and lifted up by a cotton wool tip. Injections (fig. 1) were carried out in two different groups to test for differences in local spread and the formation of local extrapancreatic tumors due to tumor cell spread. One group received 2.5×10^5 viable tumor cells in 20 μ l of ice-cold PBS. The second group received 2.5×10^5 viable tumor cells in 20 μ l of ice-cold Matrigel™ (BD Bioscience, San José, Calif., USA). Matrigel is a substance with a more watery consistency at 4°C but turns into a gel-like substance when reaching temperatures >22°C. It was used to prevent local spread and thereby the early development of local peritoneal tumor growth.

Tumor cells were slowly injected into the head of the pancreas using a pre-cooled 27-gauge needle and a pre-cooled calibrated special syringe (Hamilton Syringe, Reno, Nev., USA). To further prevent leakage, a cotton wool tip was pressed onto the injection site for 30 s. To ensure the same injection technique for all experiments, the injection was always carried out by the same person (M.S.). The pancreas was then placed back into the abdominal cavity. The abdominal cavity was closed by a running single-layer 4-0 polyester suture (Catgut, Markneukirchen, Germany). To decrease postsurgical pain and the effects of surgery, we subcutaneously injected buprenorphine hydrochloride at a dose of 0.1 mg/kg. So far, we have not had any fatalities related to surgery.

Observation Period, Assessment of Tumor Size and Survival

All mice were checked daily for their general condition and twice weekly for tumor formation. In case of severe tumor disease or another form of mice were sacrificed by ether narcosis and cervical dislocation. Necropsies were carried out on all of these animals immediately after sacrifice. Sites of obvious or potential tumor growth, including the pancreas, the liver, peritoneal lymph nodes as well as other suspicious tissue, were harvested and routinely processed for histologic microscopic examination.

Ten and 21 days after tumor injection, animals were scanned for tumor growth using a 7-tesla small-animal MRI scan. Survival analyses were performed using the Kaplan-Meier procedure.

7-Tesla Small-Animal MRI

For MRI experiments, anesthesia was induced by isoflurane (1–1.5%). Thereafter, all mice were kept in a supine position. High-power-field, 7.0-tesla MRI for small animals (Bruker, ClinScan, 7.0 T, 290 mT/m gradient strength; Karlsruhe, Germany) was performed and analyzed in a whole mouse body coil (Bruker) using a T₂-TSE sequence. The whole body coil is a volume coil and allows imaging of the whole mouse without inducing any inhomogeneities of the signal. For the assessment of tumor size, we used high-resolution T₂-weighted images in the frontal and the horizontal plane (frontal: TR: ca. 1,200 ms; TE: 41.0 ms; FA: 180°; FoV: 42 mm × 42 mm; matrix: 240 × 320; 24 slices of 0.7 mm per slice, acquisition time: ca. 15 min; horizontal: TR: ~1,250 ms; TE: 41.0 ms; FA: 180°; FoV: 40 mm × 40 mm; matrix: 240 × 320; 24 slices of 0.7 mm per slice, acquisition time: ca. 10 min). Generated images were analyzed employing MIPAV (medical imaging processing and visualization), a special DICOM-software of the National Institutes of Health, Bethesda, Md., USA. All image slices of the tumors were marked with so-called regions of interest, which facilitated the calculation of the tumor volume.

In vitro Studies of Tumor Cells in Matrigel

Matrigel has been used in several experimental settings. Here, we assessed its role as a solvent in tumor cell injections into the pancreas to prevent local spread and thereby the early development of unintentional local peritoneal tumor growth. Matrigel is a substance with a more watery consistency at 4°C but turns into a gel-like substance when reaching temperatures >22°C. After orthotopic tumor cell injection into the mouse pancreas, it was expected to change into a gel thereby blocking the injection canal and minimizing the risk of local spread. To exclude a significant influence of Matrigel onto the viability and migration of tumor cells, we first analyzed tumor cell viability and migration in vitro. Viability was tested by simple trypan blue exclusion.

Subconfluent cultures of Panc02 or 6606PDA tumor cells were harvested as above and washed twice in ice-cold PBS. Thereafter, cells were counted and 1×10^6 cells were resuspended in 80 µl of ice-cold PBS. The cell suspension was added to 80 µl of ice-cold Matrigel solution and gently mixed for 1 min. Portions of 20 µl of this cell suspension were plated in 10-cm culture dishes, and cell adherence was allowed for 10 min in a humidified incubator at 37°C. Then, 10 ml of pre-warmed complete medium were added to these cultures. Control cultures were prepared without Matrigel. After complete adherence of tumor cells, the margin of tumor cells within the Matrigel as well as the margin of the Matrigel were marked with a marker on the outside of the dish. Similar marks were made on the non-Matrigel cultures to compare migration without Matrigel. Differences in migration were measured in millimeters and were calculated using the following formula: difference in migration = distance in migration (control) – distance in migration (Matrigel).

All experiments were done in triplicate, and results were calculated as means of all experiments. Cell migration was visualized using a fluorescence binocular microscope (Leitz, Wetzlar, Germany).

CellTiter-Blue™ Viability Assay

Cell proliferation was assessed using the CellTiter-Blue (CTB) viability assay (Promega, Madison, Wisc., USA). Pancreatic cancer cells were seeded at a concentration of 5×10^3 cells/well in 96-well plates in 100 µl of complete medium. Three duplicate wells were set up for each group. Blank wells with complete medium only served as negative controls. Proliferation was measured after 24 h by adding 20 µl of CTB reagent per well followed by a 1-hour incubation under standard cell culture conditions. Absorbance of each well was measured on a microplate fluorescence reader with an excitation of 560 nm and an emission of 590 nm.

In vitro Wounding Assay (Scratch Assay)

This test was performed largely following the protocol of Liang et al. [16]. Briefly, tumor cells were cultured until reaching confluency. Injury of the confluent monolayer was done by a scratch in the middle of the culture with a standard 200-µl pipette tip. The injured monolayers were washed twice with PBS to remove non-adherent cells. Further proliferation was inhibited by adding 1 mM of mitomycin solution (Sigma-Aldrich, Munich, Germany) dissolved in complete medium followed by a 1-hour incubation period. Wound closure was monitored using an inverted phase-contrast microscope (Leica, Wetzlar, Germany). Two independent series of experiments were performed. Migration of tumor cells into the scratched lesion, i.e. wound closure, was calculated using the formula: (X-hour value – 0-hour value)/(100 – 0-hour value) = relative migration front proportional to the initial scratch magnitude.

Histology

Tumor-bearing animals were sacrificed after the final MRI examination. The pancreas including the tumor was completely resected. The complete specimens were fixed in 10% buffered formalin, processed routinely, embedded in paraffin and cut into 4-µm sections using a microtome (Leica). Sections were then mounted onto glass slides coated with poly-L-lysine (Karl Roth GmbH, Karlsruhe, Germany), baked at 60°C for 1 h and cooled overnight. Sections were deparaffinized in xylene and washed in

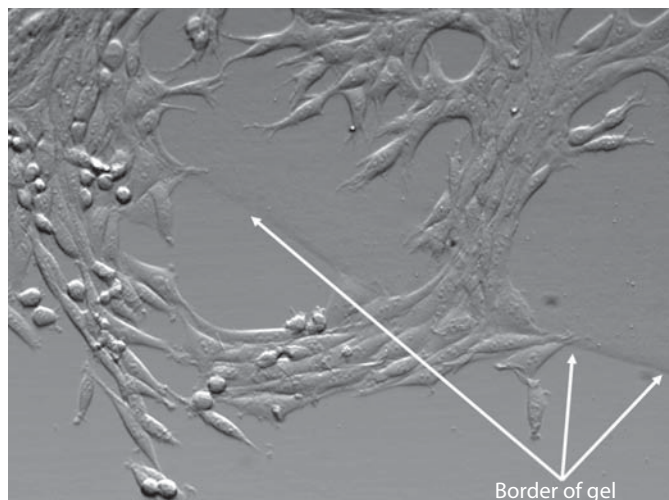


Fig. 2. Panc02 tumor cells evading Matrigel 36 h following seeding.

100% alcohol. Then, sections were washed in phosphate-buffered saline (PBS) at pH 7.4, followed by staining with hematoxylin until the desired staining was reached. Sections were rinsed in tap water for 20 min, washed in PBS followed by the second staining protocol using eosin until the desired staining was reached. Afterwards, sections were rinsed in tap water for 20 min, cleared and coverslipped.

The sections were then examined using a Leica light microscope.

Statistical Analysis

In vitro and in vivo results were statistically analyzed using the program GraphPad InStat, version 3.06 (GraphPad Software Inc., San Diego, Calif., USA). The unpaired or the paired t test with Welch's correction or the unpaired Mann-Whitney test (for non-Gaussian distribution) was employed to compare mean values of the experiments. A p value <0.05 was considered to be statistically significant.

Results

Determination of the Optimal Matrigel Concentration for in vivo Injection

In several dilution series, the optimal ratio of the PBS tumor cell suspension to Matrigel was found to be 1:1. When increasing the Matrigel, the solution became more viscous. This made the injection into the pancreatic tissue more difficult due to too fast jellification. Lower concentrations of Matrigel down to 3:1, as they are commonly used for the preparation of in vitro basal membranes, led to more fluid solutions that were not effective in pre-

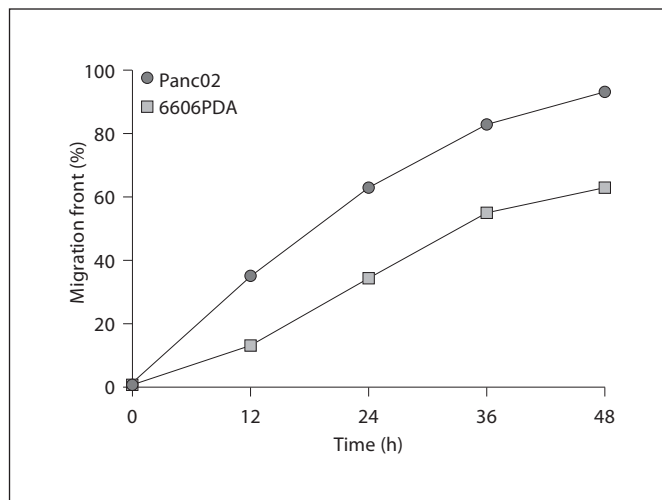


Fig. 3. Scratch assay: after 48 h, the Panc02 scratch was almost closed whereas the 6606PDA scratch was closed only about 60% (relative value).

venting leakage and local tumor cell dissemination at the injection site.

Handling of Matrigel Cell Suspensions

The handling of Matrigel itself and Matrigel tumor cell suspensions posed no difficulties as long as the Matrigel was continuously kept on ice. Injection of the ice-cold, liquid, single-cell suspension was facilitated using pre-cooled 27-gauge needles and a pre-cooled calibrated 25- μ l Hamilton syringe (Hamilton, Reno, Nev., USA).

Proliferation and Migration of Tumor Cells in vitro

To exclude a negative impact of Matrigel on tumor cells, their proliferation and migration in Matrigel were observed in vitro using a light microscope. Within 24 h, tumor cells had proliferated significantly and had already evaded the Matrigel margins (fig. 2). Further observation could not detect any negative effect of Matrigel either on proliferation or on migration of tumor cells.

Scratch Assay

Comparing migration of the cell lines Panc02 and 6606PDA, Panc02 migrated significantly ($p < 0.018$) faster into the scratch. This was clearly visible after 24 h; after 36–48 h, the scratch was completely covered. 6606PDA also showed visible migration but even after 48 h there was still a considerable distance left between the two margins of migrating tumor cells (fig. 3).

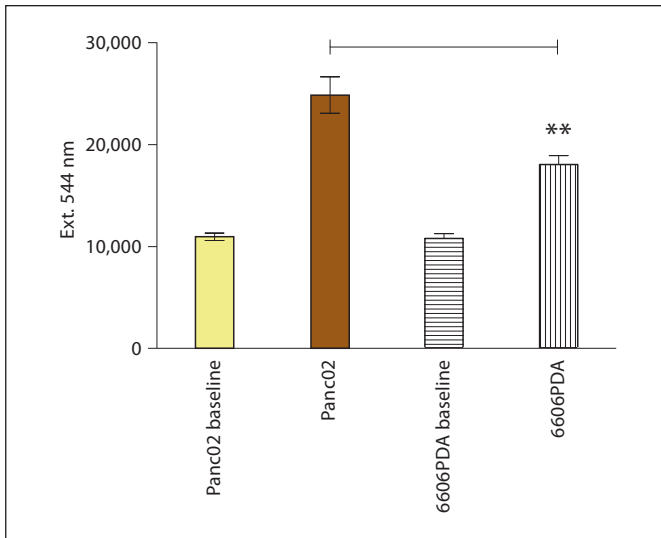


Fig. 4. Proliferation of tumor cells after 24 h of incubation (CTB assay; cell proliferation after 24 h). ** $p < 0.0071$.

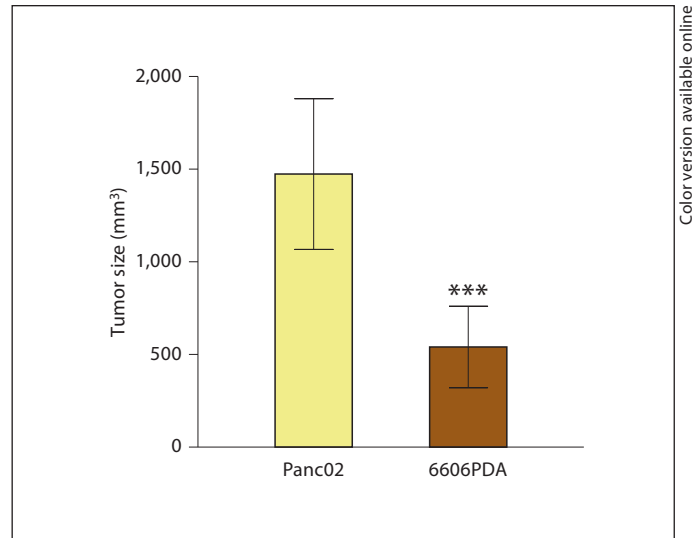
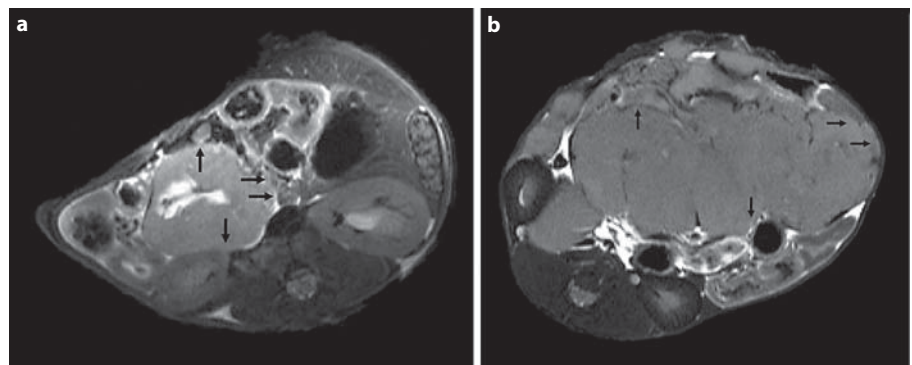


Fig. 5. Increase in tumor size 3 weeks after implantation of Panc02 versus 6606PDA (***) $p < 0.0001$.

Fig. 6. MRI scans of 6606PDA (a) and Panc02 tumors (b). Of note: 6606PDA tumors showed a more compact globe-like tumor growth often with a central zone of necrosis (a). Panc02 tumors showed a more irregular lobulated growth pattern (b) with central necroses less frequently seen than in 6606PDA tumors. Both tumors show local signs of invasion (arrows).



Proliferation Assay (CTB Viability Assay)
Panc02 cells proliferated significantly ($p = 0.0071$) faster than 6606PDA cells within 24 h: seeding of 5×10^3 tumor cells resulted in 11,346 Panc02 cells and in 8,391 6606PDA cells after 24 h of incubation, i.e. in vitro Panc02 cells grew 1.87 times faster than 6606PDA cells (fig. 4), approximating a doubling time of 18 h for Panc02 cells and 35 h for 6606PDA cells.

Tumor Growth Monitored by MRI
The pancreatic cancer cell line Panc02 developed significantly larger tumors compared to 6606PDA tumors. Ten days after tumor injection, Panc02 tumors had a mean volume of $131 \text{ mm}^3 (\pm 52 \text{ mm}^3)$ whereas 6606PDA tumors had a mean volume of $36 \text{ mm}^3 (\pm 6 \text{ mm}^3)$; 21

days after tumor injection (fig. 5), Panc02 tumors had increased to a mean volume of $1,475 \text{ mm}^3 (\pm 406 \text{ mm}^3)$. 6606PDA tumors showed mean volumes of only $540 \text{ mm}^3 (\pm 219 \text{ mm}^3)$. Also, 21 days after tumor injection, in 4 of 5 animals Panc02 tumors had induced hemorrhagic ascites. In 3 of 5 Panc02-injected mice, a distended gallbladder combined with biliary duct obstruction was found. The latter was seen only rarely in 6606PDA tumors and hemorrhagic ascites was absent in 6606PDA tumors.

Macroscopic tumor growth was also different. Panc02 showed more invasive, finger-like MRI images also displaying a more irregular disseminated pattern (fig. 6). 6606PDA MR images resembled more a spherical growth pattern, often showing a central zone of necrosis. However, both tumors were locally invasive.

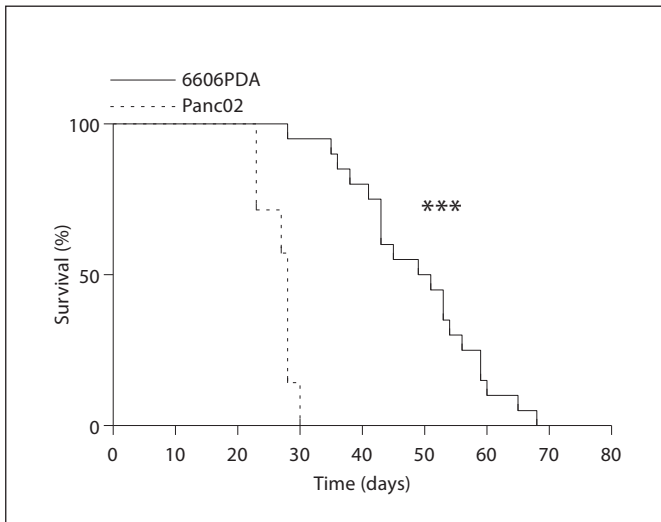


Fig. 7. Survival of mice bearing Panc02 (median 28 days) versus 6606PDA (median 50 days; *** $p < 0.0001$).

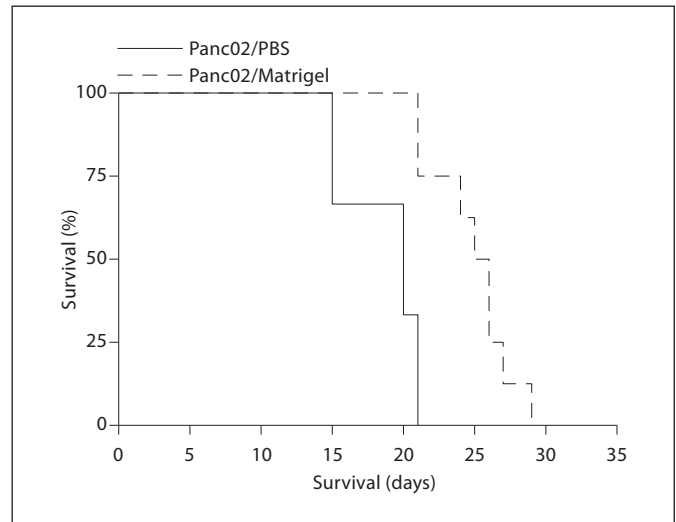


Fig. 8. Survival of mice injected with Panc02/PBS versus Panc02/Matrigel.

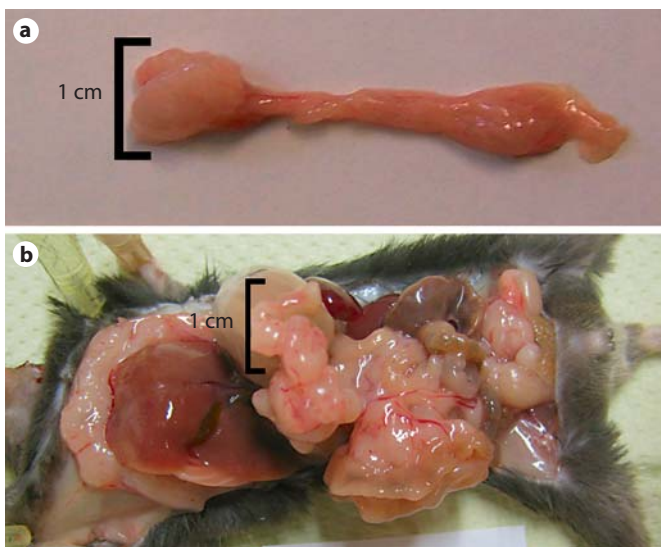


Fig. 9. Macroscopic pathology of 6606PDA (a) and Panc02 (b). Of note: 6606PDA tumors showed a more compact globe-like growth pattern (a), whereas Panc02 demonstrated a more irregular lobulated growth pattern (b).

Metastatic lesions to the liver or lung have only rarely been noticed in either tumor entity during MRI analyses (4%, i.e. in 2/50 mice). However, invasion of neighboring organs such as the liver, small and large bowel, and stomach did occur in about 30% of mice (i.e. 14/50 mice, fig. 6).

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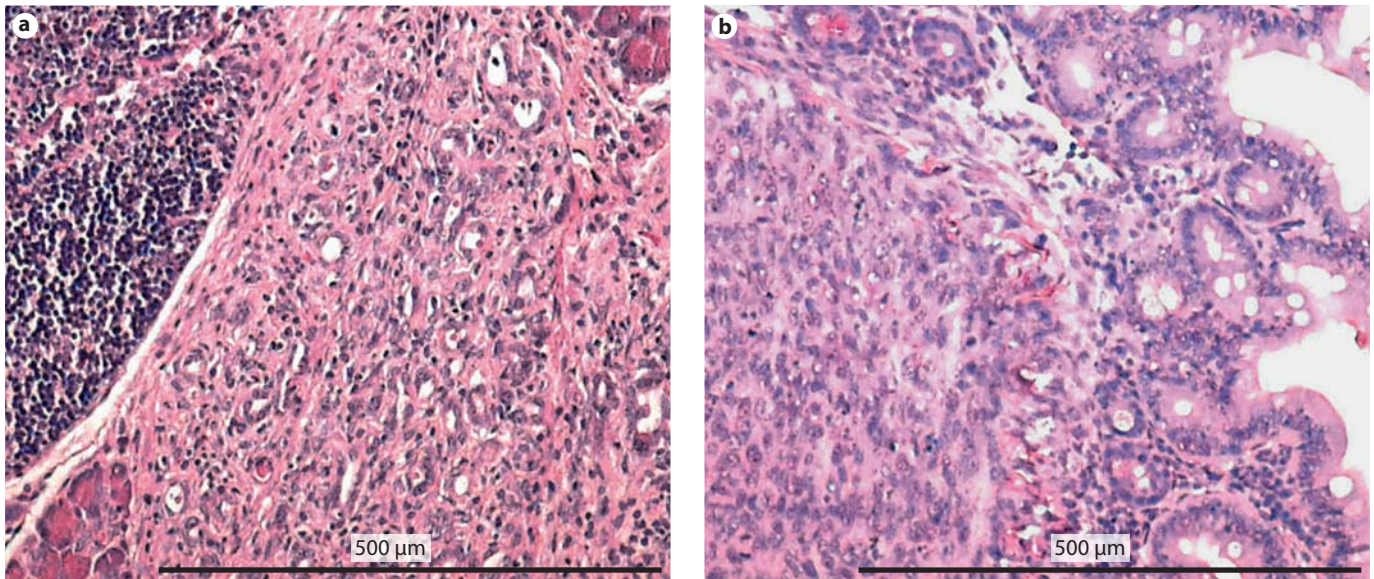
In these cases, MRI scans were highly suggestive of local tumor invasion due to the excellent high local resolution of MRI down to differences of 0.1 mm in size. This could later be confirmed by histological sections of the areas of interest.

Survival of Mice

Median survival of mice injected with Panc02 cells was 28 days (Kaplan-Meier survival curve: fig. 7). In contrast, mice injected with 6606PDA cells displayed a median survival of 50 days. This difference was statistically highly significant, $p < 0.0001$. Animals bearing Panc02 tumors usually died of local complications such as ileus and/or bile duct obstruction. This was mainly due to the rapidly growing tumor cells. In Panc02 mice without using Matrigel (fig. 8), survival was significantly shorter than in mice injected with tumor cells suspended in Matrigel (20.0 vs. 25.5 days, $p = 0.0012$). This was again due to increased peritoneal tumor spread during and/or after injection.

Macroscopic and Microscopic Pathology

Both cell lines reproducibly formed orthotopic tumors in the head of the pancreas. 6606PDA tumors displayed a more compact globe-like growth pattern whereas Panc02 showed a more irregular lobulated growth pattern (fig. 9). Both tumor lines were locally invading surrounding tissues. However, we did not detect the regular formation of



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Fig. 10. Microscopic pathology of 6606PDA (a) and Panc02 (b). Of note: 6606PDA tumor cells display a more duct-like differentiation (a) whereas Panc02 tumor cells reveal a more sarcomatous growth pattern (b). Both tumors show invasion of the surrounding tissue.

distant metastases, particularly of the liver. Lymph node metastases were often seen in both cell lines, albeit Panc02 showed lymph node metastases more frequently than 6606PDA. Microscopic analysis (fig. 10) showed moderately/well-differentiated pancreatic 6606PDA tumors with duct-like structures in many cases. These tumors resembled the growth pattern of many human pancreatic adenocarcinomas. In marked contrast, Panc02 tumors formed poorly differentiated/undifferentiated tumors displaying a sarcoma-like growth pattern. However, in both tumors strong infiltration with leukocytes could be observed.

Discussion

In this study, we present a murine orthotopic pancreatic cancer model using two different cell lines in immunocompetent C57/BL6 mice. It can be used for several purposes, e.g. to study new therapeutic agents as well as to analyze the effects of tumor growth on the immune system. At the same time, tumor growth and lymph node metastasis formation can be monitored employing a small-animal MRI.

In the past, several tumor models have been introduced for the study of pancreatic cancer. In the 1970s,

human cancer tissue was first implanted subcutaneously into nude mice. This was later followed by orthotopic cancer tissue implantation [17]. After establishing a growing number of human pancreatic cancer cell lines, these were implanted subcutaneously [18, 19] as well as orthotopically [20]. However, subcutaneous inoculation lacks many features present in human tumors, including invasive and metastatic behaviors. This is most likely a result of differences in the microenvironment in the subcutis compared with the original milieu, which also results in an altered drug response in subcutaneous tumor models [21]. Therefore, orthotopic tumor models, including orthotopic transplantation of human tumor from surgical specimens, appear to be more advantageous for preclinical studies of tumor development, metastasis formation and response to treatment. These techniques have also been used for other cancer models, including colon cancer [22]. Orthotopic transplantation enables the interaction of tumor cells with organ site-dependent microenvironmental factors, e.g. epidermal growth factor and vascular endothelial growth factor, which can modulate tumor growth patterns, progression and metastatic potential [23, 24]. However, these models still lack immune cell-tumor cell interactions since human tumor cells were transplanted as xenografts into immunodeficient mice. Syngeneic orthotopic murine tumor models avoid these

shortcomings. They appear to be the best preclinical models for studying the interaction of tumor cells with the host immune system as well as the impact of tumors on other local and systemic factors, including the surrounding tissue and the vascular system. A functioning immune system is the backbone of studies involving cytotoxic T cells, regulatory T cells, tumor-associated M2 macrophages, cell trafficking or the inflammatory fibrotic stromal reaction. Furthermore, immunocompetent models appear to be most suitable for all therapeutic studies, including studies on the route of administration of therapeutic agents [25, 26]. The development of syngeneic murine pancreatic cancer cell lines like Panc02 or 6606PDA paves the way for these models. The Panc02 cell line was established by Corbett et al. [15] by injecting 3-methyl-cholanthrene into the pancreas of C57/BL6 mice. It represents the most widely used cell line. 6606PDA cells were isolated by Tuveson et al. [27] from p48/Kras G12D mice. Both cell lines represent two different forms of differentiation. Panc02 displays a more undifferentiated growth pattern, showing a significantly more aggressive and invasive behavior than 6606PDA, which is reflected by a faster doubling time, faster migration in scratch assays and a significantly shorter survival of mice, as presented here. In contrast, the growth pattern of 6606PDA cells shows well/moderate differentiation of tumor cells on histology. Pancreatic acinus- and duct-like structures are commonly found. Yet, both cell lines show typical signs of malignant cells, e.g. uncontrolled cell growth and local invasion of surrounding tissues. It depends on the purpose of the researcher which cell line should be used in experiments. If a fast-growing, less-differentiated, more-aggressive cell is required, Panc02 is the cell line of choice. If the study requires a slower-growing, more-differentiated, less-aggressive cell, then 6606PDA is the preferred cell line. However, neither cell line has displayed the reliable formation of distant liver or lung metastases. This might be due to a possible late formation of distant metastases. In this scenario, the mouse may not live long enough to experience metastases but succumbs to the initially implanted tumor [23]. After developing murine models to resect pancreatic cancer, this issue should be further explored.

If liver metastases are required, the splenic injection technique offers a suitable model [28]. For lung metastases, the injection of tumor cells via the tail vein is an established method [19].

Subcutaneous injection of tumor cells is fairly easy to learn and perform. The injection itself can be done without any narcotics. In contrast, orthotopic injection of

pancreatic cancer cells requires a highly skilled technique. First, the implantation is only possible via laparotomy during anesthesia. Second, in contrast to the human pancreas, the murine pancreas is a soft mobile intraperitoneal organ. Third, injections into the tail, the corpus or the head of the pancreas are all feasible, but due to the size and organ texture require a skilled technique. Also, the risk of tumor cell spread after removing the injection needle is high. Tumor cell spread can cause early unwanted complications like falsely resembling peritoneal metastases. These can induce early ileus leading to an undesired bias in mortality rates. To avoid the aforementioned problems, we suggest the injection to be done by a very limited number of trained personnel, preferably by only one or two. In addition, instead of injecting commonly used liquids for tumor cell suspensions, e.g. PBS or HBSS, our results suggest to suspend tumor cells in Matrigel solutions [29]. As shown in this study, the described injection technique including the use of Matrigel significantly improved survival of mice injected with pancreatic cancer cells. This ensures reproducible orthotopic tumor growth as well as prolonged and comparable survival of mice.

Employing orthotopic tumor models has also brought up the need for *in vivo* monitoring of tumor development and possible metastasis formation. Tumors of subcutaneous models can easily be monitored by simple palpation or the use of calipers. For orthotopic tumor models, several *in vivo* murine imaging systems, including MRI, CT, CT-PET, ultrasound, as well as bioluminescence and fluorescence imaging techniques, have been developed to assess tumor size and volume [30]. These techniques have dramatically reduced the number of animals required since each animal can be scanned repeatedly in a longitudinal study, avoiding the need for large control groups. However, the MRI technique combines several advantages, making it the preferred imaging technique. MRI offers better discrimination of soft tissue and local resolution [31]. It also presents a broader spectrum of dynamically contrast-enhanced scanning options and can, in contrast to fluorescence techniques, image the complete animal [32]. Also, the experimental setup as well as the MRI technique itself allows imaging using contrast media, scanning the animal for longer periods and allowing repeated imaging series. Finally, MRI does not employ any radiation and, in contrast to ultrasonography, is less investigator dependent [33]. In the present study, tumor volumes could be determined without any problems. The detection of lymph nodes, of hemorrhagic ascites and cholestasis was possible. The high spatial resolution of MRI en-

abled us to detect changes as small as 0.1 mm. Thus, tumor infiltration, e.g. of the bowel, could be noted on MRI or MR changes were highly suggestive of tumor infiltration. These observations were confirmed by histological analyses at the end of tumor studies, and results proved that MRI is an indispensable tool for monitoring in vivo tumor growth in longitudinal studies, thus reducing the number of research animals.

In summary, the model presented closely mimics the clinical situation of human pancreatic cancer. The number of research animals can be significantly reduced in longitudinal studies. Employing two different cell lines, it can be used for imaging studies as well as non-surgical and surgical treatment purposes.

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Disclosure Statement

All authors disclose that there are no conflicts of interest.

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