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Chronic Pancreatitis and Systemic Inflammatory Response Syndrome Prevent Impact of Chemotherapy with Gemcitabine in a Genetically Engineered Mouse Model of Pancreatic Cancer[1,2]

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Abstract

BACKGROUND AND AIMS: Gemcitabine is the standard therapy for patients with pancreatic cancer with metastatic disease. Patients with metastatic pancreatic cancer presenting with increased values of C-reactive protein do not respond to gemcitabine. So far, no studies have evaluated the correlation between chronic pancreatitis, systemic inflammatory response syndrome, and the loss of chemotherapeutic benefit. *METHODS:* Pdx-1-Cre;LSL-Kras$^{G12D/+}$;LSL-Trp53$^{R172H/+}$ mice were assigned into four groups: 1) Sixteen animals received a daily intraperitoneal injection of caerulein from their ninth week of life on. 2) Sixteen mice were additionally given gemcitabine. 3) Twelve animals received gemcitabine only. 4) Saline-treated control group. Furthermore, human Paca44 pancreatic ductal adenocarcinoma cells were seeded and cultured in 0.5% FBS containing growth medium plus/minus 1 μM gemcitabine plus/minus recombinant human interleukin (IL)-6. *RESULTS:* Induced systemic inflammatory response syndrome and a mild chronic pancreatitis diminished the beneficial effects of gemcitabine upon median overall survival. In median, the monogemcitabine group survived 191 days, whereas the caerulein-mono group survived 114, the control group 121, and the caerulein gemcitabine group 127 days ($P < .05$). *In vitro*, the induction of STAT3 phosphorylation by recombinant human IL-6 promoted pancreatic ductal adenocarcinoma cell survival during gemcitabine treatment. *CONCLUSION:* We could demonstrate for the first time that an improvement in median overall survival with gemcitabine is significantly abolished by a persistent mild chronic pancreatitis and a systemic inflammatory response syndrome. In particular, the inflammation biomarkers C-reactive protein, IL-6, and IL-1α could indicate the prognostic benefit of gemcitabine chemotherapy and should now be tested in prospective patient-controlled trials.

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Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer deaths [1]. Unfortunately, the majority of patients presenting with the disease are already in an unresectable state at the time of diagnosis due to locally advanced or metastatic spread. Gemcitabine became the reference regimen for advanced pancreatic cancer after a randomized trial showed significant improvement in the median overall survival as compared with fluorouracil administered as an intravenous bolus (5.6 vs 4.4 months, $P = .002$) [2]. Numerous phase 3 trials of gemcitabine in combination with different cytotoxic or molecularly targeted agents have resulted in no substantial clinical improvement over the use of gemcitabine alone [3–8]. Only the addition of erlotinib

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to gemcitabine resulted in a significant but very small improvement in overall survival [9]. Last year, Conroy et al., demonstrated that a combination chemotherapy regimen consisting of oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX) as compared with gemcitabine as first-line therapy in patients with metastatic pancreatic cancer was associated with a survival advantage but had increased toxicity [10]. Still, the prognosis remains lethal. The efficacy of chemotherapy is still poor, and in some patients, systemic condition rapidly deteriorates after chemotherapeutic failure.

A systemic inflammatory response syndrome, marked by elevated circulating concentrations of multiple cytokines such as interleukin-6 (IL-6) or C-reactive protein (CRP), has been shown to be a disease-independent prognostic factor in a variety of tumors [11,12]. CRP is produced by the liver and is induced by proinflammatory cytokines, such as IL-6 or tumor necrosis factor-α [13], which are involved in cachexia. An elevated CRP concentration has previously been shown to have independent prognostic value in patients with PDAC [14,15]. So far, the mechanisms behind these observed phenomena are neither known nor analyzed.

Signal transducer and activator of transcription (STAT) proteins are present in the cytoplasm under basal conditions and are activated by phosphorylation on a single tyrosine residue. Activation of STAT3 depends on the phosphorylation of a tyrosine residue by upstream kinases, such as Janus kinase 2. Janus kinase 2 activation itself requires activation of the ubiquitin pathway by specific ligands, e.g., different interleukins. Of these ligands, IL-6 is one of the strongest activators of STAT3 [16]. STAT3 has been identified as a key oncogenic factor in a number of epithelial malignancies and is required for oncogenesis in mouse models of skin and gastric cancers [17,18]. In the pancreas, STAT3 is dispensable for normal development, whereas the majority of PDAC show constitutive activation of STAT3 [19].

The question remains what is the reason for patients being refractory to gemcitabine chemotherapy who present elevated CRP levels reflecting a systemic inflammatory immune response syndrome. In this study, we used a genetically engineered mouse model of PDAC which recapitulates human invasive pancreatic cancer on a genetic and histomorphologic level to address this question. We now show for the first time that gemcitabine will not have any effect on survival if a mild chronic pancreatitis and a consecutive systemic inflammatory response are induced in mice during chemotherapy. Furthermore, we found that IL-6 activates p-STAT3 which leads to increased chemoresistance in PDAC cells.

Material and Methods

Mice

Conditional *LSL-Trp53^{R172H}* [20], *LSL-Kras^{G12D}*, and *Pdx1-Cre* [21] strains were interbred to obtain *LSL-Kras^{G12D};LSL-Trp53^{R172H};Pdx1-Cre* triple-mutant animals on a mixed 129/SvJae/C57Bl/6 background as previously described [22]. All mice were generated from the same initial stock. All experiments were approved by the local committees for animal care and use. Animals were maintained in a climate-controlled room kept at 22°C, exposed to a 12:12-hour light–dark cycle, fed standard laboratory chow, and given water *ad libitum*.

Genotyping

For genotyping, genomic DNA was extracted from tail cuttings using the REDExtract-N-Amp Tissue polymerase chain reaction (PCR) kit (Sigma-Aldrich, St. Louis, MO). Three PCRs were carried out for each animal to test for the presence of the oncogenic Kras (using LoxP primers), p53, and Pdx1-Cre transgene constructs (using Cre-specific primers along with Gabra as positive control), respectively.

Drug Treatment

Transgenic Pdx-1-Cre;LSL-Kras$^{G12D/+}$;LSL-Trp53$^{R172H/+}$ mice were randomly assigned into four groups: 1) To induce a chronic pancreatitis and a consecutive systemic inflammatory response syndrome, 16 animals received a daily intraperitoneal injection of 5 μg caerulein from their ninth week of life until death. 2) Sixteen mice were additionally given 2.5 mg gemcitabine weekly from their 13th week of life until death. 3) Twelve animals received only gemcitabine from their 13th week of life until death. 4) In addition, a saline-treated group was performed as control (n = 16) (Figure 1). In cases where littermates were available for drug treatment, only the first mouse was randomly assigned to one of the three given treatment groups, the second littermate was then assigned to the "matched" control arm, and so forth to obtain the highest possible degree of consistency and to avoid randomization bias as far as possible. All mice were treated until they developed abdominal distension, reflecting the accumulation of hemorrhagic ascites, typically occurring within 48 to 72 hours before death [23].

Histologic Evaluation

After completion of drug treatment, mice were euthanized, blood was collected from the thoracic cavity for serum analysis, and pancreas and liver were removed and inspected for grossly visible tumors and metastases, and both were preserved in 10% formalin solution (Sigma-Aldrich) for histology and processed for RNA extraction (see below). Formalin-fixed, paraffin-embedded tissues were sectioned (4 μm) and stained with hematoxylin and eosin (H&E). Six sections (100 μm apart) of pancreatic and liver tissues were histologically evaluated. *LSL-Kras^{G12D};LSL-Trp53^{R172H};Pdx1-Cre* mice were classified by having developed invasive pancreatic cancer or not.

Immunostaining

For immunolabeling, formalin-fixed and paraffin-embedded archived tumor samples and corresponding normal tissues were stained as previously described [22]. Concentrations and sources of primary antibodies are available on request. Briefly, slides were heated to 60°C for 1 hour, deparaffinized using xylene, and hydrated by a graded series of ethanol washes. Antigen retrieval was accomplished by microwave heating in 10-mM sodium citrate buffer, pH 6.0, for 10 minutes. For immunohistochemistry, endogenous peroxidase activity was quenched by 10-minute incubation in 3% H_2O_2. Nonspecific binding was blocked with 10% serum. Sections were then incubated with primary antibodies overnight at 4°C. For immunohistochemistry, bound antibodies were detected using the avidin–biotin complex (ABC) peroxidase method (ABC Elite Kit, Vector Labs, Burlingame, CA). Final staining was developed with the Sigma FAST DAB peroxidase substrate kit (Sigma, Deisenhofen, Germany). Masson's trichrome was used to visualize the extracellular matrix (blue) (10×). The immunohistochemistry results were scored as described previously [24]: negative = less than 5% cells positive; + = <30% cells positive; ++ = >30% cells positive. Positive cells were counted by manual assessment within defined 10× fields of view (n = 3/section; 3 sections analyzed/animal).

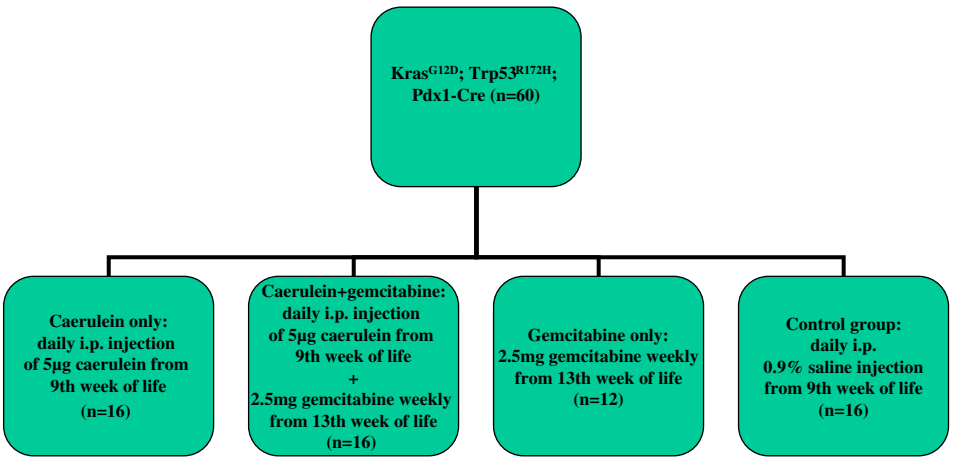

Figure 1. Study design of the LSL-Kras^{G12D}; LSL-Trp53^{R172H}; Pdx1-Cre transgenic mice.

RNA Extraction and Real-Time Reverse Transcription–PCR

A portion of fresh tumor tissue was homogenized and lysed with 600-µl buffer RLT, and whole RNA was extracted using the RNeasy kit (Qiagen, Hilden, Germany) with on-column DNA digestion following the standard protocol provided by the manufacturer. The mRNA was reverse transcribed into cDNA with oligo-dT primers using the Superscript 1st Strand System for reverse transcription-PCR (Invitrogen, Carlsbad, CA, USA) at 42°C for 50 minutes. All PCRs were carried out on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Following an activation step at 95°C for 10 minutes, determination of mRNA expression was performed over 40 cycles with 15 seconds of denaturation at 95°C and annealing/extension/data acquisition at 60°C for 60 seconds using the Power SYBR Green PCR kit (Applied Biosystems). Primer sequences are available on request. Relative fold mRNA expression levels were determined using the 2(−ΔΔCt) method. All reactions were performed in triplicates, and results are presented as means and standard errors.

Serum Analysis of mIL-1α, mIL-6, CRP, mIL-10, Interferon-γ, and Macrophage Inflammatory Protein-1α

The serum drawn from mice at the time of sacrifice was tested in commercial ELISA tests specifically according to the recommendations by the manufacturer (BD Biosciences Cell Analysis, Heidelberg, Germany) for mIL-1α, mIL-6, CRP, mIL-10, interferon-γ, and macrophage inflammatory protein-1α. The ELISA plate was measured on an Emax plate reader (Molecular Devices) and analyzed with SOFTmax Pro (Version 3-0) software.

STAT3 Phosphorylation of PDAC Cells In Vitro

Equal numbers of human Paca44 PDAC cells were seeded into six-well plates and allowed to adhere overnight. Subsequently, the subconfluent cells were washed with PBS and cultured in 0.5% FBS containing growth medium (DMEM, high glucose including sodium pyruvate, penicillin/streptomycin) plus/minus 1 µM gemcitabine plus/minus recombinant human IL-6 (20 ng/ml; Sigma-Aldrich) for 72 hours. Subsequently, the low-serum–containing medium was removed, and cells were cultured in full growth medium containing 10% FBS plus/minus IL-6 (20 ng/ml) for 6 days. Finally, cells were trypsinized, and cell numbers were determined by automated cell counting. Raw values were normalized to untreated controls. Bars represent mean values of normalized values +/− SD.

Statistical Analysis

Survival curves were computed using the Kaplan-Meier method. Log-rank test was applied to identify significant differences. Differences in the mean of two samples were analyzed by an unpaired *t* test. Comparisons of more than two groups were made by a one-way analysis of variance with *post hoc* Holm-Šidák analysis for pair-wise comparisons and comparisons versus control and by Kruskal-Wallis one-way analysis of variance. *P* values < .05 were considered statistically significant. Data were analyzed using SPSS software (Version 14; SPSS, Inc., Chicago, IL).

Results

Development of Pancreatic Cancer in LSL-Kras^{G12D}; LSL-Trp53^{R172H}; Pdx1-Cre mice

As previously described in the initial reports [21,23] and by our group [22], we observed development of fully invasive pancreatic cancers in LSL-Kras^{G12D}; LSL-Trp53^{R172H}; Pdx1-Cre transgenic mice. The histology resembled ductal adenocarcinomas of the pancreas or its precursor lesions observed in humans.

As expected by the nature of the study design, where survival was defined as the major endpoint, all mice developed fully invasive pancreatic cancer (Figure 2*A–D*). We did not find any histopathologic differences in the four groups. Furthermore, we found no differences of expression of amylase as a marker of exocrine compartment (Figure 2*E–H*) or in Masson's trichrome in between the cohorts (Figure 2*I–L*).

Induction of Systemic Inflammatory Response Syndrome and Mild Chronic Pancreatitis in Caerulein-Treated LSL-Kras^{G12D}; LSL-Trp53^{R172H}; Pdx1-Cre mice

To obtain mild chronic pancreatitis, caerulein was injected as described above. In contrast to the control group (data not shown) and the mono-gemcitabine-treated mice (Figure 3*A*), all caerulein-treated animals developed the typical changes of mild chronic pancreatitis marked by a massive infiltration of lymphocytes (Figure 3*B*).

The serum ELISA tests for mIL-6 (Figure 3*C*), mIL-1α (Figure 3*D*), and mCRP (Figure 3*E*) **were performed at the day of euthanization and** proved the systemic inflammatory response syndrome in the mice by measuring significantly elevated serum levels for these three markers in both caerulein-treated groups compared to

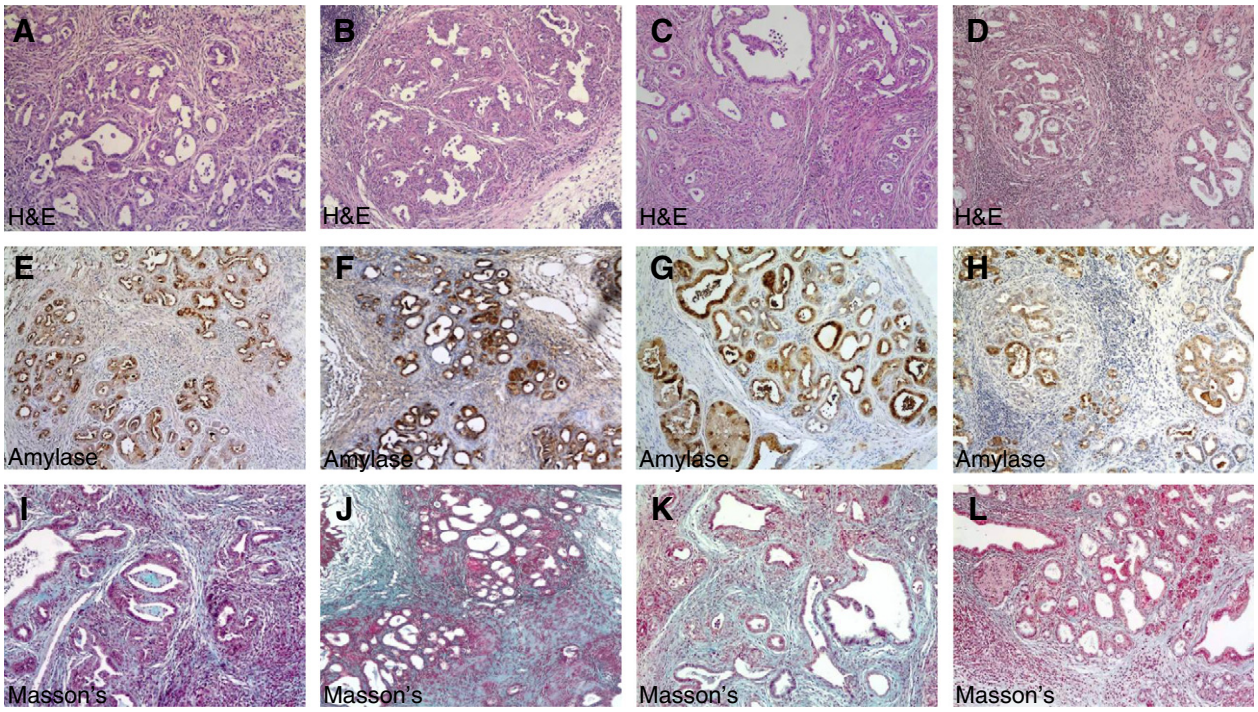

Figure 2. Development of pancreatic cancer in LSL-Kras^{G12D}; LSL-Trp53^{R172H}; Pdx1-Cre mice. All mice developed fully invasive pancreatic cancer. We did not find any histopathologic differences in the four groups (*A–D*) H&E, 10×. Furthermore, we did not find differences in expression of amylase (10×) as a marker of exocrine compartment (*E–H*) or in Masson's trichrome (10×) in between the cohorts (*I–L*).

the control group and mice treated with gemcitabine only. In contrast, IL-10, interferon-γ and macrophage inflammatory protein-1α serum levels were not significantly elevated (data not shown).

Expression of Nuclear Factor-κB (NF-κB), IL6, and p-STAT3 in LSL-Kras^{G12D}; LSL-Trp53^{R172H}; Pdx1-Cre-derived mice

After proving the induction of a mild chronic pancreatitis, we sought to evaluate the differences of expression of IL-6, p-STAT3, and NF-κB in the four different groups by immunohistochemistry (Figure 4).

IL-6 and NF-κB were widely expressed in all four groups (Figure 4*A–D* and *I–L*) without showing any significant differences. In contrast, p-STAT3 was expressed significantly higher in both groups treated with caerulein (Figure 4*F* and *H*) compared to the control group or the monogemcitabine group (Figure 4*E* and *G*).

Systemic Inflammatory Response Syndrome Diminishes the Beneficial Survival Effects of Gemcitabine in LSL-Kras^{G12D}; LSL-Trp53^{R172H}; Pdx1-Cre Mice

As expected, intraperitoneal application of gemcitabine increased median survival of *LSL-Kras^{G12D}; LSL-Trp53^{R172H}; Pdx1-Cre* mice compared to mock-treated animals (median survival 191 vs 121 days; $P < .05$; Figure 5). Induced systemic inflammatory response syndrome diminished the beneficial effects of gemcitabine upon median overall survival. In median, the caerulein-plus-gemcitabine group survived only 127 days, which was significantly less compared to the monogemcitabine group ($P = .023$). The caerulein-mono group survived 114 days.

Sonic Hedgehog (Shh) and Nestin Expression in LSL-Kras^{G12D}; LSL-Trp53^{R172H}; Pdx1-Cre Mice

Because our group previously has shown that both the hedgehog pathway and the exocrine progenitor marker nestin are activated in pancreatic injury and regeneration, we wanted to evaluate the expression of both markers in the four groups. Interestingly, we observed a stronger expression of the hedgehog ligand sonic in the stroma of mice treated with caerulein only (Figure 6*B*) compared to the other three groups (Figure 6*A*, *C*, and *D*). The same expression pattern was found for nestin, where we observed a widespread upregulation in the mesenchymal periacinar cells in the group treated with caerulein only (Figure 6*F*).

IL-6 Promotes PDAC Cell Survival During Gemcitabine Treatment

To recapitulate our *in vivo* findings in a controlled environment, we turned to cultured PDAC cells. When human Paca44 cells were grown in culture, additionally added recombinant IL-6 had a negligible effect on their growth despite the fact that they were responsive to the recombinant protein (Figure 6*I* and *inset*). On the contrary, exogenous IL-6 significantly increased the cell number in the presence of gemcitabine when compared to the treatment with gemcitabine alone. Taken together, these findings suggest that the cell growth and/or survival–promoting effects of IL-6 become most evident under conditions of cellular stress.

Discussion

The conditional *LSL-KrasG12D/+;LSL-Trp53R172H/+;Pdx-1-Cre* mice express endogenous mutant Kras and p53 alleles in pancreatic cells and develop metastatic PDAC that recapitulates the human spectrum [21,23]. The use of these models provides now the opportunity to conduct chemopreventive [22] or chemotherapeutic as well as imaging [25] studies on pancreatic cancer. Using this genetically engineered mouse model of PDAC, we could now demonstrate for the first time that the impact of chemotherapy with

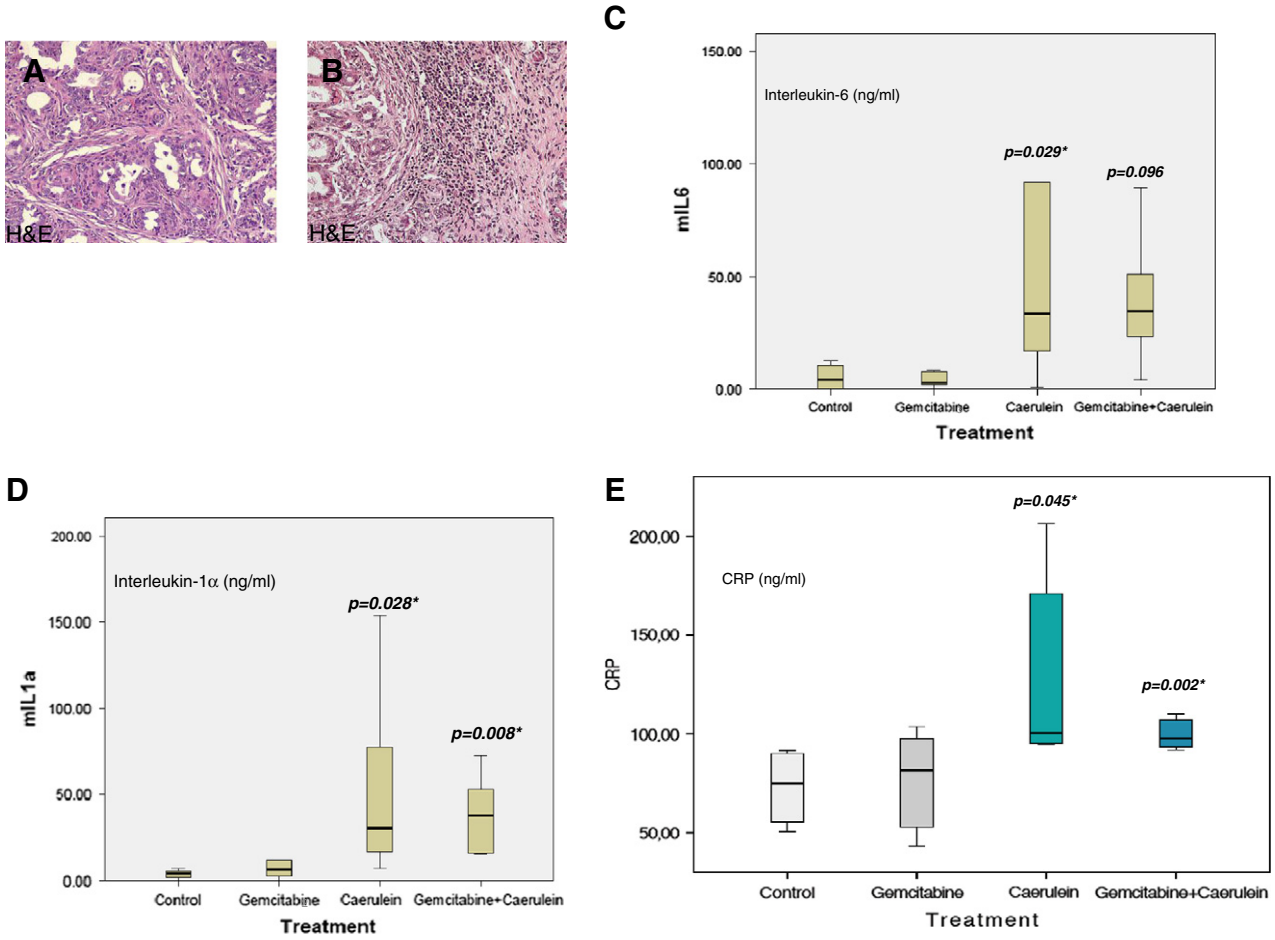

Figure 3. Mild pancreatitis and systemic inflammatory response syndrome. In contrast to the control group (data not shown) and mice treated with gemcitabine only (*A*), all caerulein-treated animals developed the typical changes of mild chronic pancreatitis marked by a massive infiltration of lymphocytes (*B*). H&E, 20×. The serum ELISA tests for mIL-6 (*C*), mIL-1α (*D*), and mCRP (*E*) proved the systemic inflammatory response syndrome in the mice by measuring significantly elevated serum levels for these three markers in both caerulein-treated groups compared to the control group and monogemcitabine group.

gemcitabine is significantly abolished by a persistent mild chronic pancreatitis and a systemic inflammatory response syndrome. To understand the idea of this study, we had to take a step back from bedside to bench.

Several clinical studies have shown for years that the presence of elevated CRP levels is a negative prognostic factor for patients with PDAC. Some years ago, a study showed that, in patients who have undergone potentially curative resection for PDAC, the presence of a systemic inflammatory response predicts poor outcome. Those patients with a postoperative CRP <10 mg/l had a median survival of 21.5 months compared with only 8.4 months in those patients with a CRP >10 mg/l (P = .001). Interestingly, patients with an elevated CRP had more vascular invasion and had poorer tumor differentiation [26]. Drawing the comparison to our study, the results from Nakachi et al., were very interesting. The authors identified patients with gemcitabine-refractory disease by univariate and multivariate analyses and found low performance status, peritoneal dissemination, and elevated CRP level to be individual risk factors. Median survival time in patients with a CRP level of <5.0 mg/dl was 2.4 months, which was significantly better than the 1.4 months for patients with CRP levels of >5.0 mg/dl [27]. In a study from Sweden [28], 119 consecutive patients with PDAC receiving palliative chemotherapy with gemcitabine were analyzed regarding predictive factors of survival. The overall median survival was 4.4 months. By means of a multivariate analysis, it was shown that CRP (P < .001) was an independent predictor of survival. The median survival of patients with normal CRP at the time of diagnosis was 10.8 months versus only 4.2 months for those with raised CRP levels (≥ = 5 mg/l; P < .001). In our study, having in mind all the difficulties of comparing mice and men, a systemic inflammatory response syndrome induced by a mild chronic pancreatitis diminished the beneficial effects of gemcitabine upon median overall survival. In median, the monogemcitabine group survived 191 days, whereas the caerulein–gemcitabine group survived only 127 days (P < .05) and was in the same range as the caerulein-mono group and the control group. **When the pancreata of the mice were resected and analyzed (Figure 2*A–D*), virtually no acinar tissue was left, leaving the question of how long during the caerulein treatment can acinar cells contribute to induce pancreatitis. In a very elegant study by Guerra et al. [29], they found that, after 3 months of caerulein treatment with the same protocol as we used in our study, mice displayed atrophic acini in about 20% and all characteristics of chronic pancreatitis. At 5 months of age, pancreata of mice displayed more severe acinar atrophy and increased fibrosis**

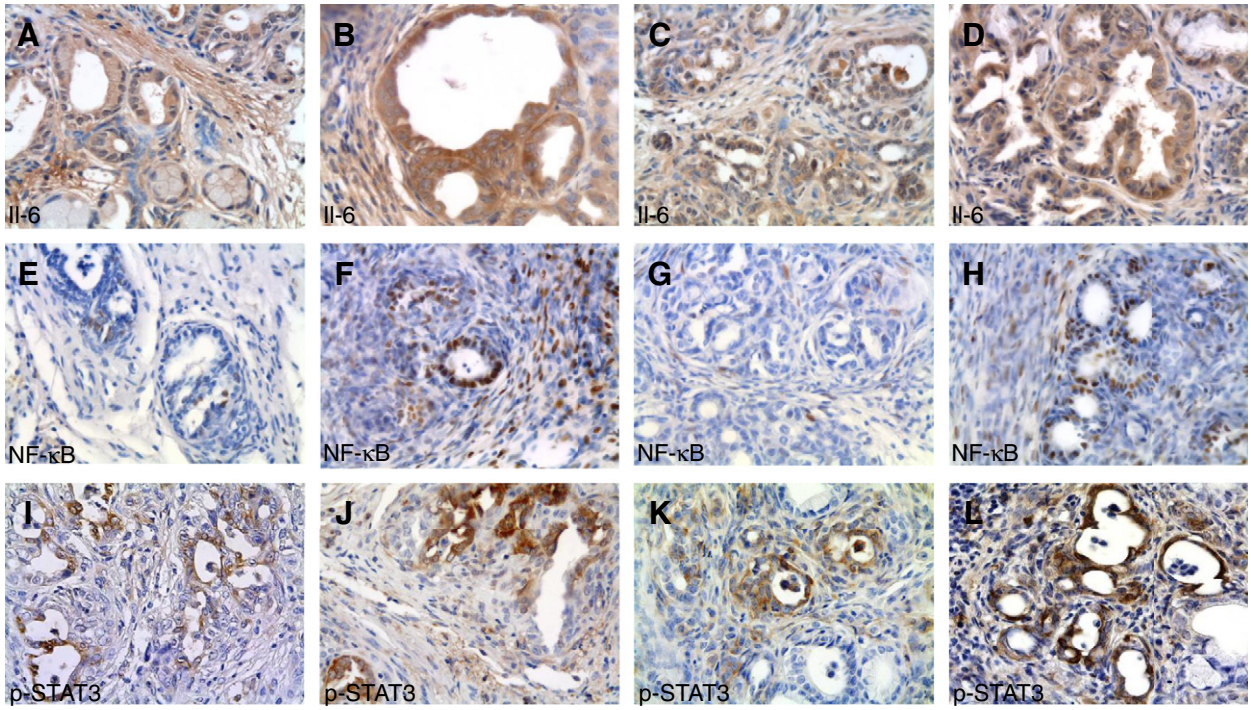

Figure 4. Immunohistochemistry for IL-6, NF-κB, and p-STAT3. IL-6 and NF-κB were widely expressed in all four groups (*A–D* and *I–L*) without showing any significant differences. In contrast, p-STAT3 was significantly higher expressed in groups treated with caerulein only or with gemcitabine and caerulein, respectively (*F* and *H*), compared to the control group or mice treated with gemcitabine alone (*E* and *G*), 40×.

beside invasive PDAC. Because, in our study, the mean survival was between 127 and 191 days, we can assume that chronic pancreatitis was maintained shortly before death of the mice.

To recapitulate our *in vivo* findings in a controlled environment, we turned to cultured PDAC cells. We found that exogenous IL-6 significantly increased the cell number in the presence of gemcitabine when compared to the treatment with gemcitabine alone. This led us to the conclusion that the cell growth and/or survival–promoting effects of IL-6 become most evident under conditions of cellular stress and may be an explanation for the lost impact of gemcitabine in our *in vivo* study. So, we next sought to evaluate the IL-6 levels in our treated mice. And indeed, we could measure significantly elevated serum levels for IL-6, IL-1α, and mCRP in mice treated with caerulein or caerulein plus gemcitabine compared to the control group or the monogemcitabine group. Because it is well known that STAT3 leads to a production of IL-6, we next evaluated the p-STAT3 levels in our resected pancreatic specimen.

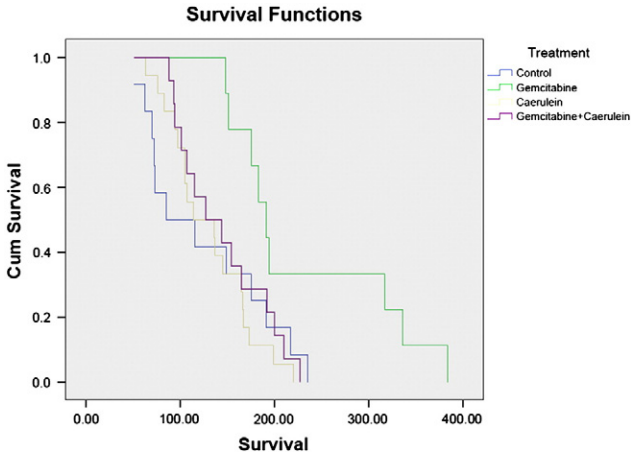

Figure 5. Systemic inflammatory response syndrome diminishes survival. Kaplan-Meier survival curve shows a prolonged overall median survival of *LSL-Kras^{G12D}; LSL-Trp53^{R172H}; Pdx1-Cre* mice treated with gemcitabine alone compared to mock-treated animals (median survival 191 vs 121 days; $P < .05$). Induced systemic inflammatory response syndrome diminished the beneficial effects of gemcitabine upon median overall survival. In median, the caerulein-plus-gemcitabine group survived only 127 days, which was significantly less compared to the gemcitabine group ($P = .023$). The caerulein-mono group survived 114 days.

In PDACs, constitutive activation of STAT3 by phosphorylation of Tyr705 has been reported in 30% to 100% of human tumor specimens, as well as in many PDAC cell lines [30,31]. By contrast, this pathway is inactive in the normal pancreas, and correspondingly, STAT3 is not required for pancreatic development or homeostasis, as shown by conditional knockout studies in mice [32]. It has been shown that STAT3 is required for the process of acinar-to-ductal metaplasia (ADM) upon ectopic expression of Pdx-1 [33]. Very recently, three elegant studies revealed the role of STAT3 in pancreatic tumorigenesis. By using the same transgenic mouse model as we did in our study, Corcoran et al., showed that STAT3 is required for the development of the earliest premalignant pancreatic lesions, ADM, and PanINs. Moreover, acute STAT3 inactivation blocked PDAC initiation in an orthotopic mouse model [19]. Two current papers demonstrate that STAT3 activation is essential for initiation and progression of PDAC [34,35]. Using the same mouse model as we did, STAT3 in pancreatic epithelial cells promotes tumor development and progression, as its ablation resulted in smaller lesions, lower tumor grade, and fewer metastases. Importantly, both groups demonstrate that STAT3 ablation in pancreatic epithelial cells attenuates *Kras*^{G12D}-induced PanIN formation, concluding

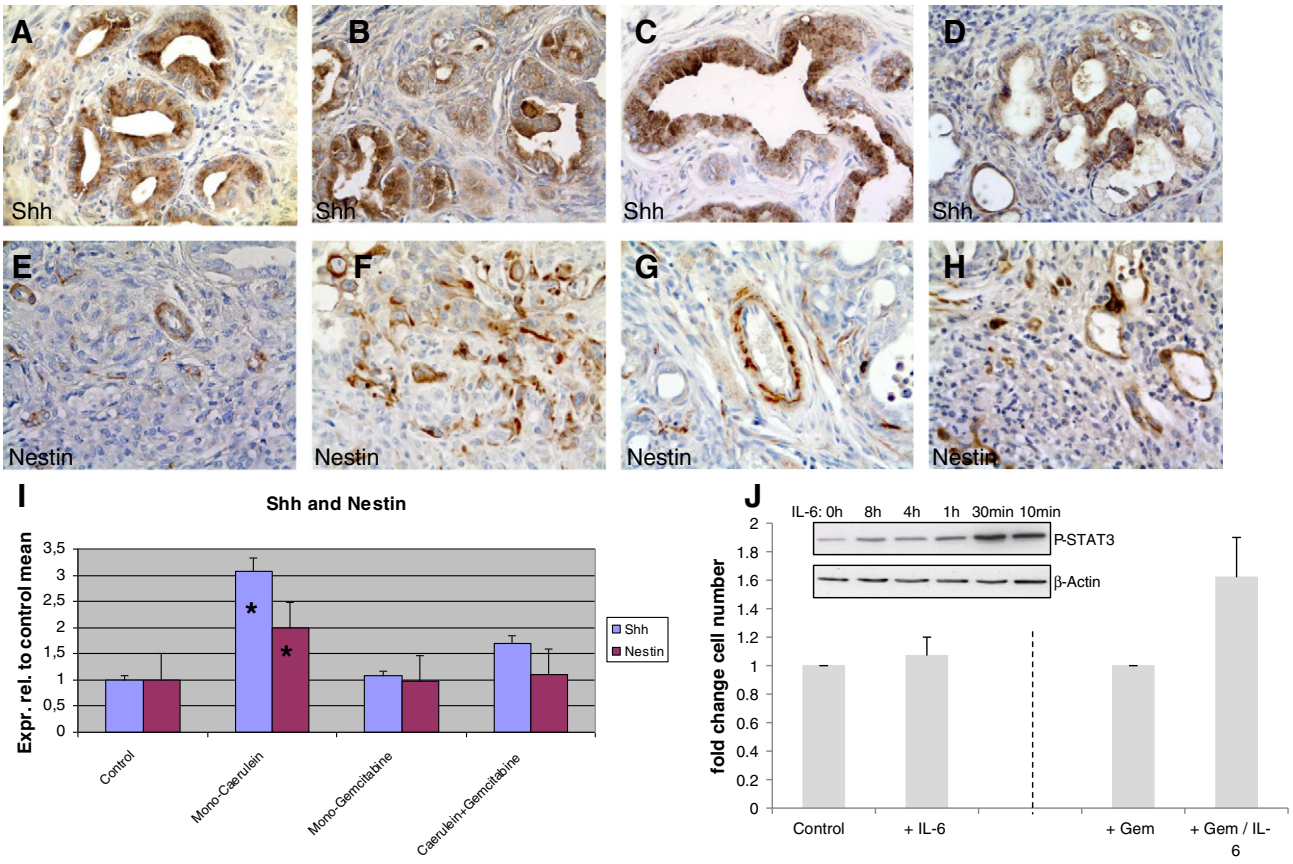

Figure 6. Immunohistochemistry for Shh and nestin. A stronger expression of the hedgehog ligand sonic in the stroma of monocaerulein-treated mice (*B*) compared to the other three groups (*A*, *C*, and *D*) was found. The same expression pattern was found for nestin, where we observed a widespread upregulation in the mesenchymal periacinar cells in the group treated with caerulein only (*F*), 40×.(*I*) **Quantitative real-time PCR on RNA obtained from treated mice demonstrates profound upregulation of Shh and nestin in the tumor (* indicates statistical significance; $P = .03$ and $P = .042$, respectively). *Error bars* indicate standard error of the mean for each measurement**.(*J*) IL-6 promotes PDAC cell survival during gemcitabine treatment. Shown is the relative change in cell number ($n = 3$; mean ± SD) upon addition of IL-6 (20 ng/ml) to control or to gemcitabine-exposed (1 μM) human PDAC cells (Paca44). For reasons of comparison, the non–IL-6 samples were both set to one (groups are separated by a dashed line).The *inset* in the upper part of the figure demonstrates the induction of STAT3 phosphorylation (Tyr 705) by recombinant human IL-6 (20 ng/ml for the indicated time periods) in Paca44 cells by means of Western blotting. β-Actin was included as loading control.

unequivocally that epithelial STAT3 is important for tumor initiation [34,35]. Interestingly, we found a significantly higher expression of p-STAT3 in groups treated with caerulein only or with gemcitabine and caerulein compared to the control group or the monogemcitabine group, a fact that is in line with very recent results from other groups [36]. Fukuda et al., found that STAT3 in pancreatic cells directly affects expression of IL-6 and IL-1, both of which are STAT3-activating cytokines [34]. This could be the reason for the elevated serum levels of IL-6 and IL-1α in our caerulein-treated mice.

Equally important is STAT3's ability to promote conversion of quiescent adult pancreatic epithelial cells to cells with a progenitor-like phenotype upon pancreatitis or other insults [34], a process often referred to as *ADM*. As a result of ADM, more ductal cells expressing progenitor cell markers, such as Pdx-1 or Shh, are formed, and these cells are more susceptible to Kras-mediated transformation [37,38]. Because our group previously has shown that both the hedgehog pathway and the exocrine progenitor marker nestin are activated in pancreatic injury and regeneration [38], we wanted to evaluate the expression of both markers in the four groups. Interestingly, we observed a stronger expression of the hedgehog ligand sonic in the stroma of mice treated with caerulein only compared to the other three groups. The same expression pattern was found for nestin, where we observed a widespread upregulation in the mesenchymal periacinar cells in the group treated with caerulein only. Both markers, especially the expression of the hedgehog ligand, may also contribute to further chemoresistance as shown by Olive et al. [20].

Which clinical impact may our preclinical study have? Unlike in advanced colorectal and hepatocellular malignancies, biologic agents have not had success in the treatment of advanced pancreatic cancer. Many patients are suitable only for palliative treatment at time of diagnosis. The decision of how aggressively to treat these patients is often difficult and depends on a number of factors, including age, medical comorbidity, cancer staging, patient preference, and local expertise. Unfortunately, in many cases, individual prognosis is unclear, and decision making can be highly subjective. Therefore, there is an important clinical requirement to select patients with biologically aggressive pancreatic cancer and to tailor both nonsurgical and surgical therapy using novel tumor- and host-based markers. In light of the mentioned clinical studies above [26–28], there is considerable interest in the systemic inflammatory response as a

prognostic factor in the cancer patient. We feel that our results strengthen these clinical observations made by these groups. In particular, the inflammation serum biomarkers CRP, IL-6, and IL-1α could indicate the prognostic benefit of gemcitabine chemotherapy and should now be tested in prospective patient-controlled trials.

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