SHORT COMMUNICATION

Loss of Periostin Results in Impaired Regeneration and Pancreatic Atrophy after Cerulein-Induced Pancreatitis

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The extracellular matrix molecule periostin (POSTN, encoded by *POSTN*), which is secreted by activated pancreatic stellate cells, has important functions in chronic pancreatitis and pancreatic cancer. However, the role of POSTN in acute pancreatitis and subsequent regeneration processes has not been addressed so far. We analyzed the function of POSTN in pancreatic exocrine regeneration after the induction of a severe acute pancreatitis. *Postn*-deficient mice and wild-type control animals received repetitive cerulein injections, and a detailed histologic analysis of pancreatic tissues was performed. Although there was no difference in pancreatitis severity in the acute inflammatory phase, the recovery of the exocrine pancreas was massively impaired in *Postn*-deficient mice. Loss of *Postn* expression was accompanied by strong pancreatic atrophy and acinar-to-adipocyte differentiation, which was also reflected in gene expression patterns. Our data suggest that POSTN is a crucial factor for proper exocrine lineage-specific regeneration after severe acute pancreatitis. (*Am J Pathol* 2016; 186: 24–31; <http://dx.doi.org/10.1016/j.ajpath.2015.09.022>)

The exocrine compartment of the pancreas consists mainly of digestive enzyme-secreting acinar cells, which possess a remarkable regeneration capacity after tissue damage in mice. Mouse models of acute pancreatitis (AP), induced by the application of the cholecystokinin analog cerulein, for instance, have revealed that acinar cells are able to transiently undergo acinar-to-ductal metaplasia (ADM) and harbor progenitor-like features. These are defined by reexpression of pancreas progenitor genes and increased cell proliferation to compensate the tissue damage.¹ Within a few days, the exocrine compartment of the murine pancreas fully recovers. AP is furthermore accompanied by a strong immune cell infiltration and a transient desmoplastic reaction, whereas the islet cells are not affected by cerulein-mediated pancreatic injury.¹ It is generally assumed that interactions among the different cell compartments contribute to a successful regeneration process.² Although we are aware of the regeneration capacity in mice, circumstantial evidence of pancreatic regeneration in humans also exists but requires further investigation.^{3,4} Understanding the molecular

and cellular processes in restoring pancreatic exocrine cell homeostasis is crucial because an impaired regenerative program can lead to insufficient organ recovery, loss of function, and potentially carcinogenesis.

The extracellular matrix (ECM) protein periostin (POSTN, encoded by *POSTN*), also termed osteoblast-specific factor-2, is a 90-kDA secretory protein that belongs to the fasciclin family. It functions as adhesion molecule and mediates its effects through binding to the integrin receptors $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_6\beta_4$.^{5,6} Initially, it was identified to be expressed in the periosteum and periodontal ligament of mice, playing an important role in bone and tooth formation and in their maintenance.^{7,8} The expression level of POSTN in healthy adult tissues is

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very low, whereas in a variety of inflammatory diseases, such as fibrosis, arthritis, atherosclerosis, and chronic allergic skin inflammation, its expression is highly up-regulated.^{9–11} In chronic pancreatitis (CP), high expression of periostin could be detected in perilobular and interacinar areas, and α -smooth muscle actin (Acta2, hereafter α -SMA)—positive myofibroblasts, derived from pancreatic stellate cells, are prominently present.^{12,13} We have formerly reported, for the first time to our knowledge, that pancreatic stellate cells are the only source of POSTN secretion in the human pancreas.¹⁴ An excessive desmoplastic reaction is also a typical characteristic of pancreatic ductal adenocarcinoma in which POSTN is strongly expressed around tubular complexes within the activated stroma at the tumor-normal tissue interface.¹⁵ Although many studies have investigated the epithelial-mesenchymal compartment interaction in AP or CP and in pancreatic ductal adenocarcinoma, there are still open queries that need to be addressed.

In this study, we investigated whether the expression of a single mesenchymal cell—derived ECM molecule might affect epithelial cell behavior during regenerative processes in the pancreas. Therefore, we analyzed the consequences of Postn ablation in adult mice after the induction of severe AP. Our results indicate that the absence of POSTN strongly impairs the regeneration of the pancreatic exocrine compartment and influences the acinar cell fate.

Materials and Methods

Mice

Postn global knockout mice (B6;129-Postn^{tm1Jmol/J}), referred as *Postn*^{−/−} mice, were purchased from The Jackson Laboratory (Bar Harbor, ME)¹⁶ and housed under pathogen-free conditions at the Technical University Munich (Munich, Germany). For all experiments, age- and sex-matched littermates were used and B6129 wild-type (WT) mice (The Jackson Laboratory) served as controls. All experiments were approved and performed in accordance with the regulations of the government of Bavaria, Germany. Pancreas mass and weight of the mice were weighed with a scale and the ratio calculated. Three to five mice were used in each experimental group.

Induction of Pancreatitis

AP was induced by eight hourly i.p. injections of cerulein (Sigma-Aldrich, Steinheim, Germany) dissolved in 0.9% saline (100 μ g/kg) (Braun, Melsungen, Germany) administered on 2 consecutive days in 8-week-old, sex-matched mice. After different time points (1, 2, 4, 7, and 21 days), five mice per group were sacrificed and tissue was taken for subsequent analyses.

Hematoxylin and Eosin Staining and Histopathologic Scoring

Paraffin sections were deparaffinized in Roticlear (Merck, Darmstadt, Germany), rehydrated, and stained with

hematoxylin and eosin (Merck) according to standard conditions. Photographs were taken with a Zeiss Axioskop 40 microscope (Zeiss, Jena, Germany), and histologic scoring was performed by two experienced pathologists (K.S. and A.-M.S.) blinded to the experimental setup. Pancreatic damage and regeneration were quantified by scoring one representative slide per mouse using whole tissue sections from three to five mice per group. ADM was specified in percentage of the whole pancreatic tissue section. Edema, stromal activation, lipomatosis, and pancreatic atrophy were scored (0 to 3). Details of the scoring system are modified from Schmidt et al¹⁷ and listed in [Supplemental Table S1](#).

Immunohistochemistry

Immunohistochemical (IHC) staining was performed according to standard conditions using the following antibodies: periostin (1:200; Acris, Herford, Germany), BrdU (1:500; Cell Signaling, Beverly, MA), α -amylase (1:5000; Santa Cruz Biotechnology Inc, Santa Cruz, CA), CD45 (1:10; BD Pharmingen, San Diego, CA), and insulin (1:400; HyTest Ltd, Turku, Finland). For IHC analysis, primary antibody was omitted to serve as a negative control. In case of BrdU staining, tissue sections of mice without BrdU injection were used. As positive control for CD45 staining, IHC with splenic tissue was performed. As positive control for POSTN staining, pancreatic cancer tissue exhibiting a strong stromal response was used. For quantification at least three high-power fields per slide were manually counted or evaluated using the Axiovision program (Zeiss).

Activated Stroma Index

The activated stroma index (ASI) was assessed as previously described.¹⁸ Briefly, consecutive sections were stained using the Aniline dye (Sigma-Aldrich) for collagen deposition and using immunostaining against α -SMA (Dako, Hamburg, Germany) for activated myofibroblasts. Without any counterstaining, the slides were scanned (Nikon Scanner, Tokyo, Japan), and stained areas were quantified using Photoshop elements CS5 (Adobe Systems Inc, San Jose, CA).

Quantitative Real-Time PCR

Pancreatic tissue from cerulein-treated and untreated control WT and *Postn*^{−/−} mice was harvested, and RNA was immediately extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) and transcribed into cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, St. Leon-Rot, Germany). Quantitative real-time PCR was performed with the SYBR Green I Master Mix (Roche Diagnostics, Penzberg, Germany) on a LightCycler 480 instrument (Roche). Relative quantification of gene expression and normalization to WT controls was calculated

Figure 1 Characterization of pancreatitis in wild-type (WT) and periostin (*Postn*)-deficient mice. **A:** *Postn* protein levels are shown as representative immunohistochemistry staining in WT mice during acute pancreatitis and regeneration (**top panel**) and the mRNA expression in a graph below. The **bottom panel** shows the localization of *Postn* expression in acute pancreatitis. In early acute pancreatitis (D1), *Postn* is expressed in the interlobular and intralobular area (**left panel**) and the periacinar area (**right panel**). **B:** Representative hematoxylin and eosin staining of WT and *Postn*^{-/-} mice after induced acute pancreatitis on D1, D2, D4, D7, and D21. **C:** Representative images of α -amylase staining 21 days after the last cerulein injection in WT and *Postn*^{-/-} mice reveal impaired regeneration of the exocrine compartment in *Postn*^{-/-} mice. Quantification of α -amylase positive areas confirms impaired regeneration in *Postn*^{-/-} mice. **P* < 0.05. Scale bars: 100 μ m (**A, top**; **A, bottom left**; **B**; **C**); 20 μ m (**A, bottom right**). D, day.

with the $\Delta\Delta$ CT method as described earlier.¹⁹ The following primer sequences were used: housekeeping gene *Ppib* forward: 5'-GGAGCGCAATATGAAGGTGC-3', *Ppib* reverse: 5'-CTTATCGTTGGCCACGGAGG-3'; *Hes1* forward: 5'-AAAATTCCTCCTCCCCGGTG-3', *Hes1* reverse: 5'-TTTGGTTTGTCCGGTGTCTG-3'; *Pdx1* forward: 5'-TGC-CACCATGAACAGTGAGG-3', *Pdx1* reverse: 5'-GGAA-TGCGCACGGGTC-3'; *Sox9* forward: 5'-GCAAGCTGG-CAAAGTTGATCT-3'; *Sox9* reverse: 5'-GCTGCTCAG-TTCACCGATG-3'; *Mist1* forward: 5'-CCCCAGTTGGA-AGGGCCTCA-3', *Mist1* reverse: 5'-TCCTGCATGGGT-GTTCGGCG-3'; *Rbpjl* forward: 5'-GTATCGAAGTCAG-TGGCGGT-3', *Rbpjl* reverse: 5'-GCAGGCTCAGGTGA-GTCAA-3'; peroxisome proliferator-activated receptor gamma (*PPAR* γ) forward: 5'-GAAAGACAACGGACAA-ATCACC-3', *PPAR* γ reverse: 5'-GGGGGTGATATGTT-TGAACTTG-3'.

Statistical Analysis

Data are presented as means $\pm$ SEM. Statistical significance among control (WT), *Postn*^{+/-}, and *Postn*^{-/-} was determined by the nonparametric *t*-test or Mann-Whitney *U* test using GraphPad Prism software version 5 (GraphPad Software Inc, San Diego, CA). *P* < 0.05 was considered statistically significant.

Results

Up-Regulated Expression of Periostin during AP

As an initial step we examined the expression levels of *Postn* in AP and the subsequent regeneration phase in WT mice. Therefore, we used our standard cerulein application model and harvested the pancreas at days 1, 2, 4, 7, and 21 after the last cerulein injection. Untreated WT mice served as initial controls (day 0). IHC staining against *Postn* revealed no protein expression in the healthy organ of 8-week-old WT mice. However, a strong *Postn* expression could be observed in the acute inflammatory phase at days 1 and 2, and it was gradually reduced in the regeneration phase at days 4 and 7. Fully regenerated pancreatic tissue at day 21 revealed nearly no *Postn* expression (**Figure 1A**). Similar observations could be found on the mRNA level, where *Postn* exhibited a comparable expression profile over time (**Figure 1A**). In the inflammatory phase at days 1 and 2, *Postn* was localized interlobularly and intralobularly, although the periacinar *Postn* expression was more dominant as shown in a high-power image (**Figure 1A**). On days 4 and 7, *Postn* was mainly restricted around ADM and regenerative areas (**Figure 1A**). Likewise as in CP, *Postn* expression was strongly induced throughout the fibrotic reaction in cerulein-mediated AP. Although CP reveals a

Figure 2 Periostin ablation impairs regeneration. **A:** Pancreas mass/body weight ratio reveals significant differences between wild-type (WT; gray bars) and periostin (*Postn*) global knockout mice (*Postn*^{-/-}; white bars) at D7 and D21. **B:** Amount of edema is elevated at all time points in *Postn*^{-/-} mice compared with WT mice. **C:** Scoring of lipomatosis (left graph) and pancreatic atrophy (right graph) during regeneration of WT and *Postn*^{-/-} mice reveals that recovery is severely impaired in *Postn*^{-/-} mice. Images show representative hematoxylin and eosin staining of pancreatic atrophy and lipomatosis 21 days after the last cerulein injection. **D:** Quantification of CD45⁺ cells in WT and *Postn*^{-/-} mice during acute pancreatitis and regeneration shows a persistent high immune cell infiltration in *Postn*^{-/-} mice at D7 and D21. Representative immunohistochemical staining for CD45 at D21 is shown. **P* < 0.05, ***P* < 0.01. Scale bars: 100 μ m (**C** and **D**). D, day; HPF, high-power field.

persistent *Postn* expression, in AP it diminished in the recovery phase.

Depletion of Periostin in AP

To determine the functional role of increased *Postn* expression in AP, we used a transgenic global periostin knockout mouse model (*Postn*^{-/-}) for further experiments. Gene expression data confirmed the lack of *Postn* expression (Supplemental Figure S1A), and the *Postn*^{-/-} mice had no morphologic changes of the healthy pancreas. At 8 weeks of age the exocrine compartment, which mainly consists of acinar cells, appeared histologically normal as indicated by α -amylase staining. Similarly, insulin immunostaining for the endocrine compartment revealed no changes compared with WT control mice (Supplemental Figure S1B). We furthermore examined the pancreas/weight ratio and found no differences in relation to WT mice (Supplemental Figure S1C). These data indicate that loss of *Postn* expression during embryogenesis has no obvious influence on pancreatic organ development.

Next, we induced severe AP according to our standard cerulein model and harvested the pancreatic tissue of *Postn*^{-/-} mice at corresponding time points. A general histologic examination was performed and revealed a comparable level of severity in the acute phase at days 1 and 2. Both mouse models had infiltrating immune cells, stromal activation, and ADM formation. Later time points, however, clearly exhibited alterations in the regeneration phase. At day 4, *Postn*^{-/-} mice had

persistent ADM areas, infiltrated immune cells, and stromal reaction, whereas by this time the acinar cells of WT mice were almost recovered. Particularly, the effects were more striking at day 7 and day 21, when *Postn*^{-/-} mice had a heavily impaired exocrine regeneration with parenchymal atrophy (Figure 1B). We also induced AP in *Postn*^{+/-} mice and sacrificed the mice at corresponding time points. Histologic analysis revealed that *Postn*^{+/-} mice had a similar phenotype as WT mice. However, in contrast to the WT mice, *Postn*^{+/-} mice had a slight form of pancreatic atrophy and lipomatosis starting from day 7 (Supplemental Figure S2). To quantify the regeneration of the exocrine compartment, we performed immunostaining for α -amylase and measured positive stained areas. Calculation of the α -amylase-positive areas revealed a typical stepwise increase of α -amylase-expressing cells due to pancreatic regeneration in WT mice. However, *Postn*^{-/-} mice had a lack of acinar cell recovery and a persistent reduction of α -amylase-positive areas from day 2 on compared with WT mice, which had significant differences at day 21 (Figure 1C). These data indicate that the depletion of the ECM molecule *Postn* might directly influence exocrine acinar cell recovery after cerulein-mediated pancreatitis.

Detailed Histologic Evaluation of *Postn*^{-/-} Mice

To further analyze the pancreatic atrophy in *Postn*^{-/-} mice, the overall pancreas mass/weight ratio was determined and compared with that of WT mice. These data indicated that

Figure 3 Analysis of the stromal compartment. **A:** Consecutive sections of wild-type (WT) and periostin (*Postn*) global knockout mice (*Postn*^{-/-}) were stained for hematoxylin and eosin, α -smooth muscle actin (α -SMA), and aniline. Scanned representative pictures of WT and *Postn*^{-/-} mice at D2 are shown. **B:** Scoring of stromal activation of WT (gray bars) and *Postn*^{-/-} (white bars) mice. The activated stroma index (ASI) was calculated and is shown for pancreatitis and recovery. Both analyses reveal a higher stromal activation in *Postn*^{-/-} mice in acute pancreatitis and regeneration. **C:** The amount of acinar-to-ductal metaplasia (ADM) in WT and *Postn*^{-/-} mice revealed that *Postn*^{-/-} mice had more ADM at all time points. Representative hematoxylin and eosin staining of WT and *Postn*^{-/-} mice 7 days after the last cerulein injection. **D:** Analysis of proliferation in WT and *Postn*^{-/-} mice reveals a higher proliferation rate in *Postn*^{-/-} mice. Representative BrdU staining at D7 of WT and *Postn*^{-/-} mice. * $P < 0.05$, ** $P < 0.01$. Scale bars: 20 μ m (C); 100 μ m (D). Original magnification, $\times 50$ (A). D, day; HPF, high-power field.

the pancreas mass of *Postn*^{-/-} mice decreased at each time point and was significantly reduced at days 7 and 21 (Figure 2A). Moreover, a detailed histologic scoring of cerulein-treated WT and *Postn*^{-/-} mice was performed for all time points. No significant difference was found in regard to the amount of edema between *Postn*^{-/-} and WT mice (Figure 2B). However, further scoring of histopathologic features revealed a fatty replacement of the pancreas parenchyma in *Postn*^{-/-} mice starting at day 7 and reaching significance at day 21, when nearly the whole exocrine pancreas compartment was replaced by fat cells. In parallel, *Postn*^{-/-} mice had pancreatic atrophy from day 4 on with significant differences compared with WT mice at days 7 and 21 (Figure 2C). To further assess whether *Postn* ablation affects the inflammatory response, we analyzed the number of infiltrating immune cells over time. Quantification of CD45⁺ cells revealed no differences in immune cell infiltration in the inflammatory phase at days 1 and 2. However, in the

regenerative phase, significant differences could be observed. *Postn*^{-/-} mice had a persistent high immune cell infiltration during the recovery phase, which was most distinctive at day 21 (Figure 2D).

Analysis of the Stromal Compartment and ADM Formation

After acute inflammation of the pancreas, fibroblasts and pancreatic stellate cells get transiently activated and produce ECM proteins.²⁰ The ECM protein *Postn* plays an important role in keeping pancreatic stellate cells in this activated state and thus contributing to the abundant ECM deposition and fibrotic reaction.¹⁴ Consistent with these observations, *Postn* depletion results in reduced fibrosis in various other pathologic conditions.^{21–23} Hence, we determined whether *Postn* influences the stromal response after cerulein-induced tissue damage. We therefore calculated the ASI as

Figure 4 Dysregulated expression of progenitor, differentiation, and adipogenesis markers in periostin (*Postn*) global knockout mice (*Postn*^{-/-}). **A:** mRNA expression levels are shown for the pancreatic progenitor markers Hes1, Sox9, and Pdx1 and the differentiation markers Mist1 and Rbpjl in wild-type (WT) and *Postn*^{-/-} mice at D21. *Postn*^{-/-} mice had an up-regulated expression of progenitor and a reduced expression of differentiation markers at D21 compared with WT mice. **B:** Representative immunohistochemical staining of α -amylase in a *Postn*^{-/-} mouse at D21 reveals α -amylase-containing granules in the cytoplasm of adipocytes (black arrows). Analysis of the key adipogenesis regulator peroxisome proliferator-activated receptor gamma (PPAR γ) in WT and *Postn*^{-/-} mice at D21 reveals a significant up-regulation of this marker in *Postn*^{-/-} mice. **P* < 0.05, ***P* < 0.01. Scale bar: 20 μ m.

previously described by our group²⁴ and additionally examined the stromal compartment by histologic evaluation. Both analyses found an elevated stromal activation characterized by a higher amount of α -SMA-positive cells and simultaneous lower collagen deposition in *Postn*^{-/-} mice at all time points with particular differences at days 2 and 7 (Figure 3, A and B). These data indicate that loss of the ECM protein Postn results in less deposition of collagen and thus in reduced fibrosis in the inflammatory and regenerative phases.

To further examine the effect of Postn ablation on acinar cell metaplasia and cell recovery in AP, we evaluated ADM in our mouse models. ADM is a prerequisite for acinar cell regeneration, where acinar cells regain proliferation capacity to compensate for the cerulein-derived damage. Afterward, they differentiate back to acinar cells during tissue renewal.¹ Here, *Postn*^{-/-} mice had strongly elevated levels of ADM at nearly all time points, particularly at day 7, indicating that the lack of Postn expression provokes an impairment of acinar cell recovery and redifferentiation (Figure 3C). Because of the higher abundance of ADM in *Postn*^{-/-} mice, we next analyzed cell proliferation because we anticipated higher rates of proliferating cells in *Postn*^{-/-} mice as well. Indeed, quantitative analysis of BrdU-positive cells exhibited an increased number of proliferating cells in *Postn*^{-/-} mice at all time points with a significant difference at day 7. However, at day 21 in both mouse models, nearly no proliferating cells could be detected, which can be explained

by a fully regenerated pancreas in WT mice and hardly no present acinar cells in *Postn*^{-/-} mice (Figure 3D).

Dysregulated Regenerative Program

In addition to the proliferation capacity, ADM-forming acinar cells also transiently reexpress pancreatic progenitor genes. The expression, however, decreases after successful pancreatic recovery in which the acinar cells regain their specific expression of differentiation genes.^{1,25} Because of persistent ADM formation and discovered acinar atrophy in *Postn*^{-/-} mice, we examined the expression of pancreatic progenitor and differentiation markers at day 21. Quantitative real-time PCR analysis revealed a significant up-regulation of the pancreatic progenitor markers Hes1, Sox9, and Pdx1 in *Postn*^{-/-} mice at day 21 compared with WT mice. Accordingly, mRNA expression levels of the differentiation markers Mist1 and Rbpjl were strongly down-regulated in *Postn*^{-/-} mice compared with WT mice at day 21 (Figure 4A).

Because the severely impaired pancreatic regeneration of *Postn*^{-/-} mice was particularly characterized by the enormous replacement of acinar cells by fat tissue, we carefully assessed the IHC staining of α -amylase at day 21. We observed α -amylase-positive fat cells, indicating a process of acinar-to-adipocyte differentiation in *Postn*^{-/-} mice (Figure 4B). We furthermore analyzed the expression of the adipogenesis key regulator PPAR γ in WT and *Postn*^{-/-} mice. As expected, WT mice had hardly any expression

of PPAR γ whereas in *Postn*^{-/-} mice a significant up-regulation of transcripts could be observed (Figure 4B).

Discussion

The ECM molecule periostin, which is secreted by activated pancreatic stellate cells, has important functions in many inflammatory diseases and in pancreatic cancer.^{5,9,10,14} However, its role in severe AP has not been investigated yet. Using global periostin knockout mice, our study found that lack of periostin does not overtly influence embryonic pancreatic development, although we did find that it is essential for regeneration of the exocrine pancreatic compartment after severe AP. To our knowledge, this is the first study to report that an extracellular molecule is crucial for acinar differentiation commitment. So far, only intrinsic factors, such as c-Myc and Gata6, have been identified to be indispensable for acinar differentiation.^{26,27}

The finding that periostin expression is up-regulated in the acute phase of pancreatitis and that its expression decreases gradually during the regeneration phase indicates that periostin has an important function in contributing to tissue homeostasis after cerulein-induced pancreatitis. Indeed, we did not observe any differences in severity of pancreatitis, whereas periostin deficiency resulted in a massively impaired regeneration characterized by persistent high immune cell infiltration, elevated levels of ADM, and pancreatic atrophy. These results are consistent with a recently published study that described increased periostin expression during regeneration of WT mice after partial pancreatectomy and impaired regeneration of the β -cell compartment in periostin-deficient mice.²⁸ Furthermore, these findings are consistent with studies that found that periostin is essential for successful regeneration of various other organs after tissue injury.^{29–32}

The most striking phenotype of *Postn*^{-/-} mice in our study is the replacement of acinar cells by adipocytes starting 7 days after the last cerulein administration. Usually, acinar cells undergo a restricted program after tissue damage. They transdifferentiate into ductlike cells, reexpress pancreatic progenitor markers, and exhibit a proliferative phenotype to replace the injured organ whereby they finally differentiate back to mature acinar cells.^{1,25} In *Postn*^{-/-} mice, we observed that this process is heavily disturbed. We found elevated mRNA levels of the pancreatic progenitor markers Hes1, Pdx1, and Sox9 and decreased levels of acinar differentiation markers Rbpjl and Mist1 21 days after the last cerulein injection, indicating that the acinar cells remained in an undifferentiated state. Because we additionally discovered α -amylase-positive fat cells and a significant up-regulation of the key adipogenesis regulator PPAR γ , we postulate that in these mice acinar-to-adipocyte differentiation occurs because it was previously described in mice lacking c-Myc or Gata6 expression.^{26,27}

To confirm acinar-to-adipocyte differentiation as a major source of the abundant accumulation of fat, lineage tracing would be necessary because an additional mesenchymal origin of adipocytes cannot entirely be excluded.

In conclusion, we described a completely novel function of periostin—that the ECM molecule was apparently associated with the regulation of acinar cell fate decision and restoring pancreatic tissue integrity. Because it is still not fully clear how periostin influences acinar differentiation, further studies are needed to clarify the underlying molecular mechanisms. Nevertheless, our study emphasizes the importance of an epithelial-stromal crosstalk for successful regeneration of the exocrine pancreatic compartment.

Acknowledgments

S.H., I.R., M.E., and J.K. conceived and designed the study; S.H., N.W., and M.T. performed experiments; S.H. and I.R. analyzed the data; I.E. and C.W.M. advised in experimental setup and data analysis; K.S. and A.-M.S. scored H&E staining; S.H. and I.R. wrote the manuscript; and I.E., C.W.M., H.F., J.K., and M.E. revised the manuscript, which was read and approved by all authors.

Supplemental Data

Supplemental material for this article can be found at <http://dx.doi.org/10.1016/j.ajpath.2015.09.022>.

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