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ORIGINAL RESEARCH

Isolation of Murine Pancreatic Stellate Cells and the Establishment of a New ex-vivo Activation Model

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Background: Pancreatic stellate cells (PSCs) are critical in the development of pancreatic fibrosis. In vitro, cell attachment itself can promote cell activation. Currently, there is a lack of methods for isolating activated PSCs that are unaffected by cell attachment. This study aims to identify effective methods for isolating quiescent and activated murine PSCs (mPSCs) and to evaluate the potential of caerulein in inducing mPSC activation in an ex vivo model.

Methods: Pancreatic tissue from mice was digested with collagenase P (1.17 U/mL), Pronase (0.5 mg/mL), and DNase I (0.01 mg/mL). Quiescent and activated mPSCs were isolated using a Nycodenz gradient. Immunostaining for α-smooth muscle actin (α-SMA), Desmin, glial fibrillary acidic protein (GFAP), vimentin, CK19, and CD68 was performed to confirm cell purity. Real-time quantitative PCR (RT-PCR) and RNA sequencing assessed the activation phenotype following caerulein treatment.

Results: Quiescent and activated mPSCs were successfully isolated using the Nycodenz gradient, with cells exhibiting typical stellate morphology and positive staining for α-SMA, Desmin and vimentin. Oil Red O staining confirmed lipid droplets in quiescent mPSCs. In the caerulein-treated group, mPSC activation was significantly greater than in the saline-treated control group. RT-PCR revealed progressive upregulation of acta2 (**p<0.01, d4 compared to d2, $^{\#\#}p$<0.01,d7 compared to d4,**p<0.01,d7 compared to d2), col1a (**p<0.01, d4 compared to d2,**p<0.01,d7 compared to d2), and actg2 (**p<0.01, d4 compared to d2, $^{\#\#}p$<0.01,d7 compared to d4, **p<0.01,d7 compared to d2) mRNA levels at 2, 4, and 7 days post-adhesion. Fibroblast markers were also upregulated, and KEGG and GO enrichment analyses identified key pathways involved in ECM-receptor interactions, cell cycle regulation, PI3K-Akt signaling, and extracellular matrix remodeling.

Conclusion: The Nycodenz gradient efficiently isolates quiescent mPSCs, and short-term caerulein treatment effectively activates mPSCs ex vivo, providing a valuable model for studying mPSC activation and related signaling pathways.

Keywords: pancreatic stellate cells, chronic pancreatitis, pancreatic fibrosis

Introduction

Chronic pancreatitis (CP) is a progressive and irreversible fibroinflammatory disease characterized by recurrent episodes of acute pancreatitis and persistent inflammation.[1] Ultimately, pancreatic fibrosis elevates the risk of developing pancreatic cancer, a condition associated with high mortality and significant disease burden.[2,3] Pancreatic stellate cells (PSCs) are widely recognized as a key precursor in the process of pancreatic fibrogenesis.[4] Alpha-smooth muscle actin (α-SMA), Desmin and

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vimentin are usually used as markers for PSCs. The abnormal activation of PSCs under pathological conditions disrupts extracellular matrix (ECM) homeostasis, leads to ductal destruction, and induces extensive interstitial fibrosis, finally results in chronic pancreatic failure characterized by diminished endocrine and exocrine functions.[1] Consequently, as a critical hallmark of CP, more attention needs to be paid to the mechanisms underlying PSCs activation.

Considerable progress has been made with respect to the understanding of the characteristics of PSCs over the past few decades. Various protocols for isolating primary PSCs from rat and human pancreatic tissues through outgrowth and density gradient centrifugation have been reported.[5–7] However, these methods described in the literature presented several limitations and exhibited considerable variability. Although the PSCs grow from the tissues are abundant, their activation by culture plates renders them unsuitable for studies focused on their quiescent state. Effective protocols for harvesting PSCs from rats via ladder centrifugation have been reported. However, a precise and appropriate method for isolating quiescent mouse PSCs (mPSCs) is lacking.

In our study, we aimed to outline a method involving the mixed digestion of Collagenase P, Pronase and DNase I, followed by density gradient centrifugation method for isolating PSCs from murine pancreatic tissue. Furthermore, the activation of PSCs plays a central role in the context of pancreatic fibrosis. Previously published research has strongly emphasized the importance of cytokine transforming growth factor beta (TGF-β) in the driving of PSCs activation in vitro.[8,9] However, TGF-β is not the only key signal driving the activation events; hypoxia, alcohol, oxidation stress, platelet-derived growth factors (PDGF) and many other factors coordinate in the activation and proliferation of PSCs.[10,11] Caerulein, an analogue of cholecystokinin (CCK), stimulates excessive secretion from pancreatic acinar cells, promotes the activation of mPSCs and leads to the excessive self-digestion of pancreatic tissue, enabling it a common model for acute and chronic pancreatitis.[12] Also, the pathological fibrotic changes induced by caerulein in the pancreas closely resemble those observed in patients with chronic pancreatitis. Hence, in this study, we proposed to explore a new ex-vivo model of quiescent and activated mPSCs through short-term intraperitoneal caerulein injection, aiming to better explore the activation patterns and physiological roles of mPSCs.

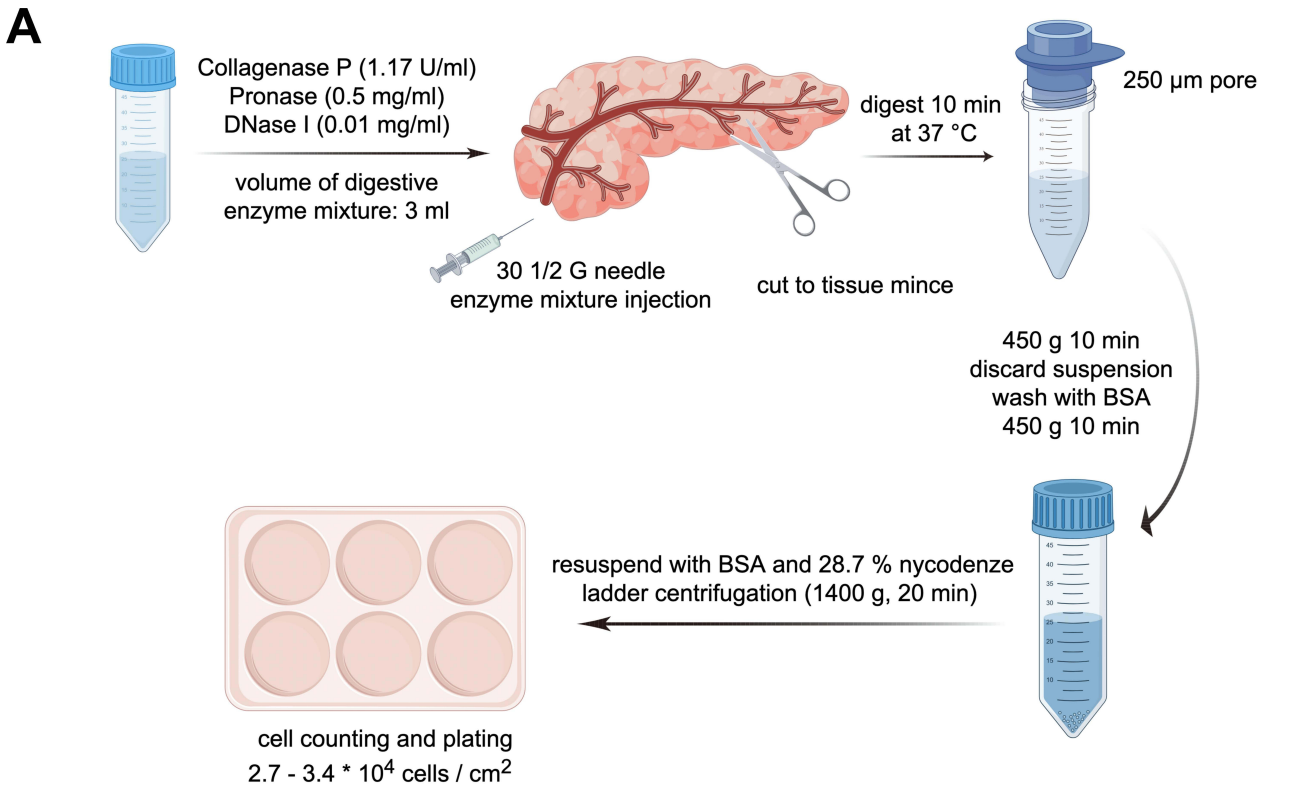
Materials and Methods

Materials

Dulbecco's modified eagle medium with Ham's F12 medium (DMEM/F12) and fetal bovine serum (FBS) were purchased from Gibco (Carlsbad, CA, USA); Collagenase P, Pronase and DNase I were purchased from Roche (Castle Hill, Australia); bovine serum albumin (BSA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The anti-α-SMA antibody, anti-Desmin antibody, anti-vimentin antibody, anti-CD68 antibody and anti-Cytokeratin 19 antibody were purchased from Abcam (Cambridge, United Kingdom), the anti-glial fibrillary acidic protein (GFAP) antibody was purchased from Cell Signaling Technology; Alexa Fluor® 594 mouse anti-goat immunoglobulin G (IgG), Alexa Fluor® 488 rabbit anti-goat IgG and DAPI were purchased from ZSGB-BIO (Beijing, China); normal mouse IgG and normal rabbit IgG were purchased from Beyotime (Shanghai, China). Nycodenz was purchased from Serumwerk Bernburg AG; Caerulein was purchased from Topscience (Shanghai, China).

Isolation Methods of Primary mPSCs

Five 6-8-week-old C57BL/6J mice were anesthetized via intraperitoneal injection of 0.1% pentobarbital and subsequently disinfected with 30 minutes of UV light. The abdominal skin and peritoneum were incised using tissue scissors, taking care not to damage the intestines. An enzyme mixture solution (consisting of Collagenase P (1.17 U/mL), Pronase (0.5mg/mL) and DNase I (0.01mg/mL)), 0.3% BSA and 28.7% Nycodenz solution were prepared with Gey's balanced salt solution (GBSS) with or without salt. The enzyme mixture was warmed to 37 °C before use, and the Nycodenz solution must be kept in the dark. The isolation methods are presented in Figure 1A. After clamping the common bile duct with a vascular clamp, the preheated digestive enzyme was slowly injected into the biliopancreatic duct using a 30 1/2 G needle (3mL per mouse) (Figure 1B and C). The fully swollen and digested pancreatic tissues were pooled in a centrifuge tube containing 10 mL of enzyme solution and shaken for 10 minutes at 37 °C. Tissue fragments were further minced with tissue scissors. After neutralizing with complete medium and filtering through a 250 μm nylon mesh, the digested tissues were washed with BSA



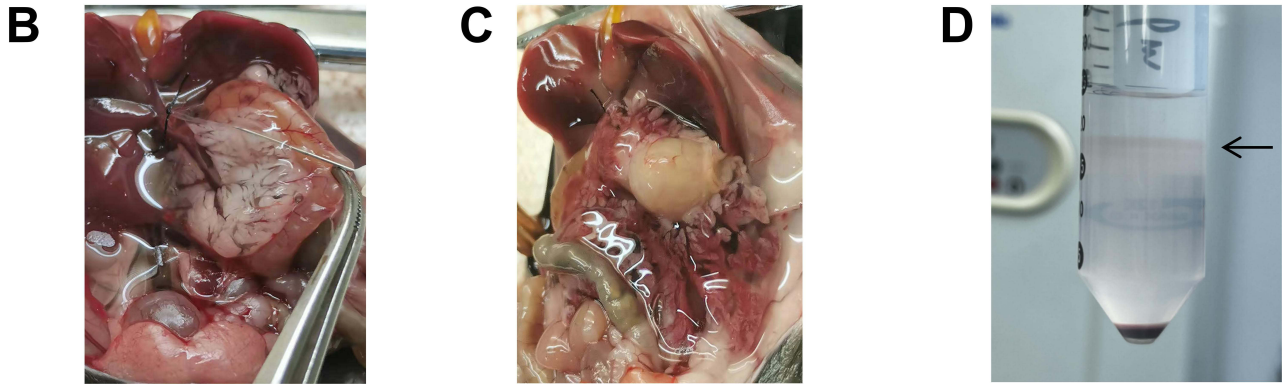


Figure 1 Isolation methods of primary mPSCs. (**A**) The flow chart of ladder centrifugation methods for mPSCs. (**B**) The enzyme mixture solution was injected into the biliopancreatic duct of murine. (**C**) The pancreatic tissue was fully swollen and digested. (**D**) The white and thin cell layer in the middle isolated by ladder centrifugation was mPSCs.

and centrifugated at 450 × g for 10 minutes. The cell suspension was then mixed with 9.5 mL of BSA and 8 mL of 28.7% Nycodenz. 6 mL of BSA was prepared in another 50 mL centrifuge tube, and the Nycodenz gradient was created by layering the cell suspension underneath BSA, taking care not to disrupt the interface. The gradient solution was then centrifuged at 1400 × g for 20 minutes at 4 °C. The white, thin cell layer (Figure 1D) was harvested and washed with BSA at 450 × g for 10 minutes at 4°C to obtain purified mPSCs. The cells were then resuspended in DMEM/F12 medium containing 10% fetal bovine serum and antibiotics (1% penicillin and streptomycin). Trypan blue staining indicated the proportion of living cells was greater than 90%, with cells primarily exhibiting a small, round shape. Finally, 0.8 −1.3 × 10^6 mPSCs were obtained from a mouse pancreatic tissue weighing 200–250 mg. The cells were then resuspended in complete medium and seeded at a density of 2.7–3.4×10^4 cells / cm^2 for further study.

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Activated mPSCs Model in vivo

In this study, an activated mPSCs model was established by intraperitoneal injection of caerulein (50 μg/kg) on the first day and the third day with 6 timely a day respectively. Normal saline (NS) was administered to serve as the quiescent control group. The mPSCs in both groups were isolated the day after the last injection.

Immunofluorescence and Oil Red O (ORO) Staining

For immunofluorescence, primary mPSCs were isolated by ladder centrifugation and cultured on rat tail collagen-coated 1cm^2 glass coverslips in 12-well plates. After 2 days of adherent culture, glass coverslips were washed in PBS (pH 7.4) to remove medium proteins and then fixed in 4% paraformaldehyde for 20 minutes. Thereafter, cells were preincubated for 20 minutes in 0.2% TritonX-100, followed by blocking for 60 minutes in PBS with 3% bovine serum albumin (BSA). Primary mouse monoclonal antibodies (α-SMA, GFAP and CK-19) and rabbit monoclonal antibodies (Desmin, vimentin and CD68) were incubated at working dilutions at 4°C in a humid chamber for 12 hours. Nonspecific staining was controlled by using mouse or rabbit IgG respectively at working dilutions. Next, after rinsing cells 5 minutes for 3 times with PBS, the second antibody (anti-mouse or anti-rabbit, both diluted 1:100) was added and incubated for 1 hour at room temperature. Finally, DAPI was utilized to stain nucleus in the dark. Cell characteristics were observed using a fluorescence microscope. In addition, cytoplasmic fat droplets were stained using ORO. After washing and fixation, ORO staining was performed for 1 hour in the dark. The nuclei were subsequently stained with hematoxylin and examined using a microscope.

Real-Time Quantitative Polymerase Chain Reaction (RT-PCR)

The total RNA of mPSCs was extracted by TRIzol Reagent (Invitrogen, USA) and reverse-transcribed into complementary DNA (cDNA) with HiScript III RT SuperMix (Vazyme, Nanjing, China). RT-PCR was performed using PCR SYBR Green Master Mix (Yeason, Shanghai, China) in Agilent AriaMx real-time PCR system. The relative mRNA level was calculated by Livak ($2^{-\Delta\Delta Ct}$) method. The primer sequences of acta2 are as following: Forward: 5′-CCCAGACATCAGGGAGTAATGG-3′; Reverse: 5′-TCTATCGGATACTTCAGCGTCA-3′; the primer sequences of col1a1 are as following: Forward: 5′-GAGCGGAGAGTACTGGATCG-3′; Reverse: 5′- GTTCGGGCTGATGTACCAGT-3′.

RNA Sequencing

Primary mPSCs were collected by enzyme mixture digestion and density gradient centrifugation following intraperitoneal injection of caerulein or normal saline in vivo. Total RNA was then extracted from mPSCs using TRIzol Reagent, and RNA sequencing was performed at Novogene (Beijing, China). Gene set enrichment analysis (GSEA) was performed using the NovoMagic tools (https://magic-plus.novogene.com/#/), differentially expressed genes (DEGs) were identified based on |log2(Fold change) | ≥ 1 and padj value < 0.05. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of the DEGs from RNA-sequencing were also performed in the Novo Magic tools (https://magic-plus.novogene.com/#/).

Statistical Analysis

All results are presented as mean ± standard error of the mean (SEM). The normal distribution of the data was conducted. Where appropriate, statistical analysis utilized two-tailed unpaired *t*-test, Mannwetny test or Kruskal Wallis test in Prism 8 (GraphPad Software). $P<0.05$ was considered statistically significant.

Results

Verification of Isolated Cells

Primary mPSCs isolated via ladder centrifugation were cultured for adherence, and positive and negative markers were assessed to verify purity. It has been demonstrated that activated PSCs are phenotypically similar to myofibroblasts and can proliferate, acquire migratory capabilities, and produce abundant extracellular matrix proteins. The morphology of mPSCs, displayed in Figure 2A and B, exhibited an angular and flattened appearance after 2 days of culture using the ladder centrifugation method.

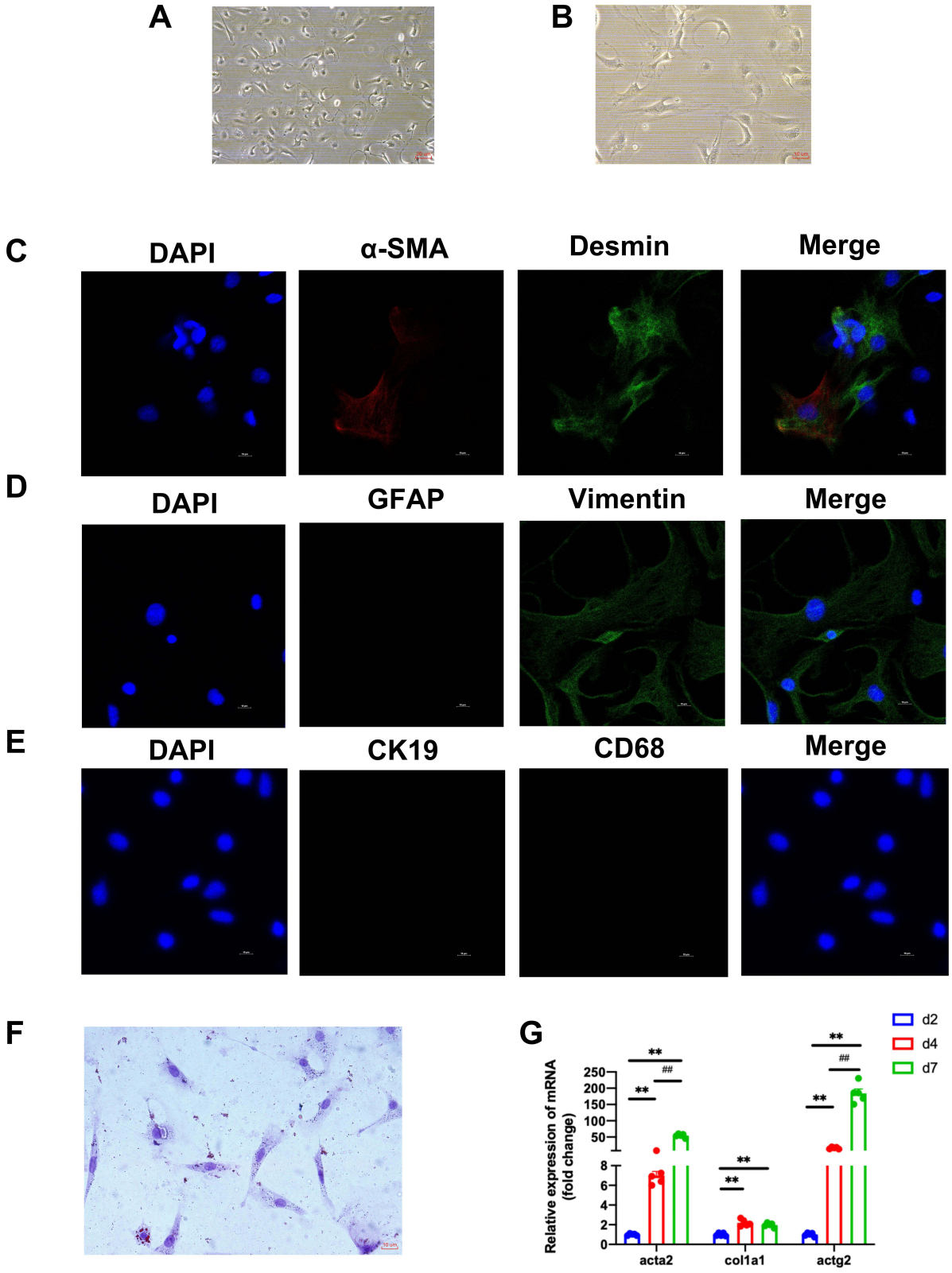

Figure 2 Verification of isolated cells. (**A**) The mPSCs isolated from ladder centrifugation method and cultured for 2 days (×100). (**B**) The mPSCs isolated from ladder centrifugation method and cultured for 2 days (×200). (**C**) Representative immunofluorescence staining images of α-SMA and Desmin in mPSCs (×400). (**D**) Representative immunofluorescence staining images of Vimentin and GFAP in mPSCs (×400). (**E**) Representative immunofluorescence staining images of CK19 and CD68 in mPSCs (×400). (**F**) The oil red O staining of mPSCs (×200). (**G**) The mRNA level of acta2, col1a1 and actg2 in isolated mPSCs cultured for 2 days, 4 days and 7 days. Data are expressed as mean ± SEM, n=5 in (**G**). (**p<0.01, compared to d2 group, $^{\#\#}p$<0.01, compared to d4 group).

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Activated PSCs specifically express α-SMA and Desmin. Cytoskeletal proteins expressed in the cytoplasm of PSCs, including vimentin and GFAP, were examined using immunofluorescence. The co-localization of α-SMA and Desmin indicated that the isolated cells were mPSCs in a relatively quiescent state (Figure 2C). Additionally, strong cytoplasmic positivity for vimentin was observed, which is associated with activated stellate cells, while GFAP showed mild positive staining (Figure 2D). These results suggest that the isolated cells are relatively quiescent mPSCs. Furthermore, epithelial cell marker CK19 and macrophage marker CD68 were also stained. The low percentages of CK19 and CD68 indicate that the isolated cells are neither epithelial cells nor macrophages, confirming the high purity of mPSCs without contamination (Figure 2E).

In addition, oil red O staining showed the lipid storage in mPSCs. In a quiescent state, mPSCs are characterized by abundant vitamin A-containing lipid droplets in their cytoplasm (Figure 2F). During activation, mPSCs gradually lose vitamin A droplets. The RT-PCR analysis of isolated mPSCs cultured for 2, 4 and 7 days showed a gradual increase in the levels of acta2 (**p<0.01, d4 compared to d2, $^{\#\#}p$<0.01,d7 compared to d4,**p<0.01,d7 compared to d2), col1a1 (**p<0.01, d4 compared to d2,**p<0.01,d7 compared to d2), and actg2 (**p<0.01, d4 compared to d2, $^{\#\#}p$<0.01,d7 compared to d4, **p<0.01,d7 compared to d2), verifying the enhanced activated characteristics of stellate cells (Figure 2G).

Short-Term Intraperitoneal Injection of Caerulein is an Effective Method to Establish mPSCs Activation Model

To establish this activated model, we adopted a different approach from the conventional method of extracting cells and subsequently inducing activation in vitro. Instead, we pre-administered caerulein intraperitoneally in vivo to induce activation. The control group received an equivalent volume of saline injection. This method allows us to obtain both quiescent and activated primary stellate cells simultaneously upon cell isolation, thereby circumventing the influence of cell attachment on activation. In this study, an activated mPSCs model was established by intraperitoneal injection of caerulein (50 μg/kg) on the first day and the third day with 6 timely a day respectively. Normal saline (NS) was administered to serve as the quiescent control group. The mPSCs in both groups were isolated the day after the last injection. The flow chart is presented in Figure 3A. Several methods were performed to verify the viability of the model. Higher cell numbers directly reflect the degree of proliferation. The number of cells in the caerulein-injected group is significantly greater than that in the saline-injected group (p<0.0001) (Figure 3B). Furthermore, after 24 hours in culture, cells in the caerulein-injected group exhibited an expanded and flattened shape compared to the saline-injected group, becoming larger and more myofibroblast-like in appearance (Figure 3C). Higher expression levels of fibroblast markers, including acta2 (p<0.05) and collagen type 1 alpha (col1a1) (p<0.05) were detected in the caerulein-treated group through RT-PCR (mRNA level) and immunofluorescence (α-SMA protein level) (p<0.05) (Figure 3D–F). In summary, we proposed a novel and effective method for isolating the activated mPSCs in vivo, constructed a new ex-vivo model. Also, harvesting mPSCs at both quiescent and activated states simultaneously enabled us to study their metabolic characteristics more conveniently and provides additional evidence for the activation mechanism of caerulein on stellate cells.

Altered mPSCs Activated Pattern was Verified by Transcriptomics Analysis

To further elucidate the activated phenotype of mPSCs isolated by our new method and the potential genes involved in the regulation of mPSCs activation, RNA sequencing was performed to compare activated and quiescent primary mPSCs. The results of principal component analysis (PCA) demonstrated a clear distribution of quiescent and activated cells, exhibiting significant reproducibility and consistency. The two major principal components explained 52.71% and 14.01% of the total variance, respectively (Figure 4A). A total of 1,190 genes were differentially upregulated in the caerulein-treated group compared to the control group, while 1,123 genes were downregulated, as screened with $|\log2FoldChange| > 1$ and $padj < 0.05$ (Figure 4B). The heatmap hierarchical clustering further showed the distinct characteristics between the two groups (Figure 4C).

Fibroblast markers indicative of a higher degree of stellate cell activation were significantly upregulated in the caerulein treated group, including Acta 1, Acta 2, the Col family, Actg2, tissue inhibitors of metalloproteinase (TIMP1), fibronectin 1 (Fn1) and Myl9. To explore the functional enrichment and annotation of differentially expressed genes

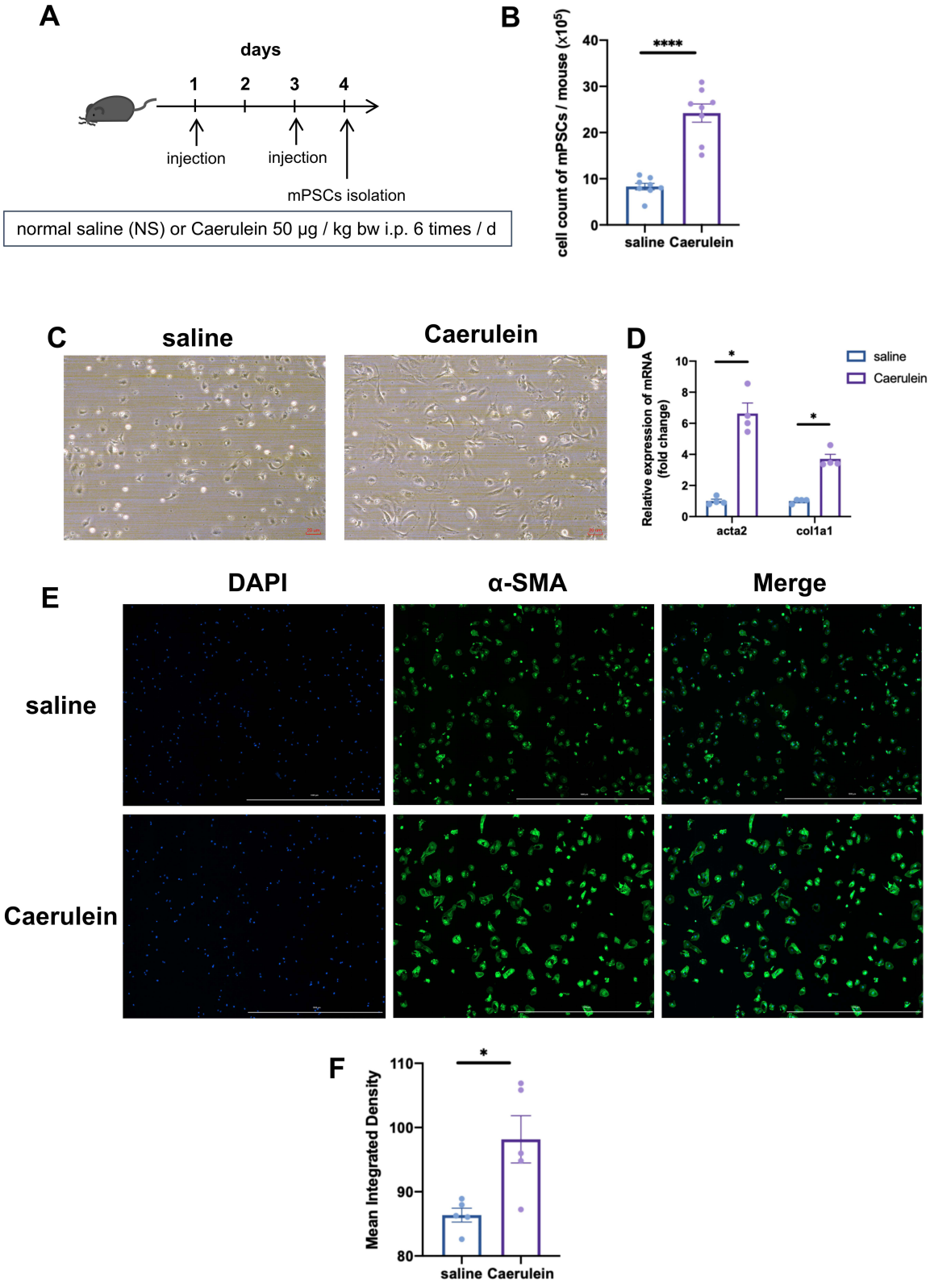

Figure 3 Short-term intraperitoneal injection of caerulein is an effective method to establish mPSCs activation model. (**A**) Activated mPSCs model was established by short-term intraperitoneal injection of caerulein or NS as control group. (**B**) The cell number of mPSCs in caerulein-injected group was greater than in NS-injected group. (**C**) The mPSCs in caerulein-injected group showed larger and flatter shape compared to NS-injected group. (**D**) The mPSCs in caerulein-injected group showed higher expression level of fibroblast markers, including acta2 and col1a1 compared to NS-injected group. (**E**) The mPSCs in caerulein-injected group showed higher level of α-SMA protein expression level. **(F)** Quantification of immunofluorescence (α-SMA). Data are expressed as mean ± SEM, n=8 in (**B**), n=4 in (**D**), n=5 in (**E**). (*p<0.05, ****p<0.0001).

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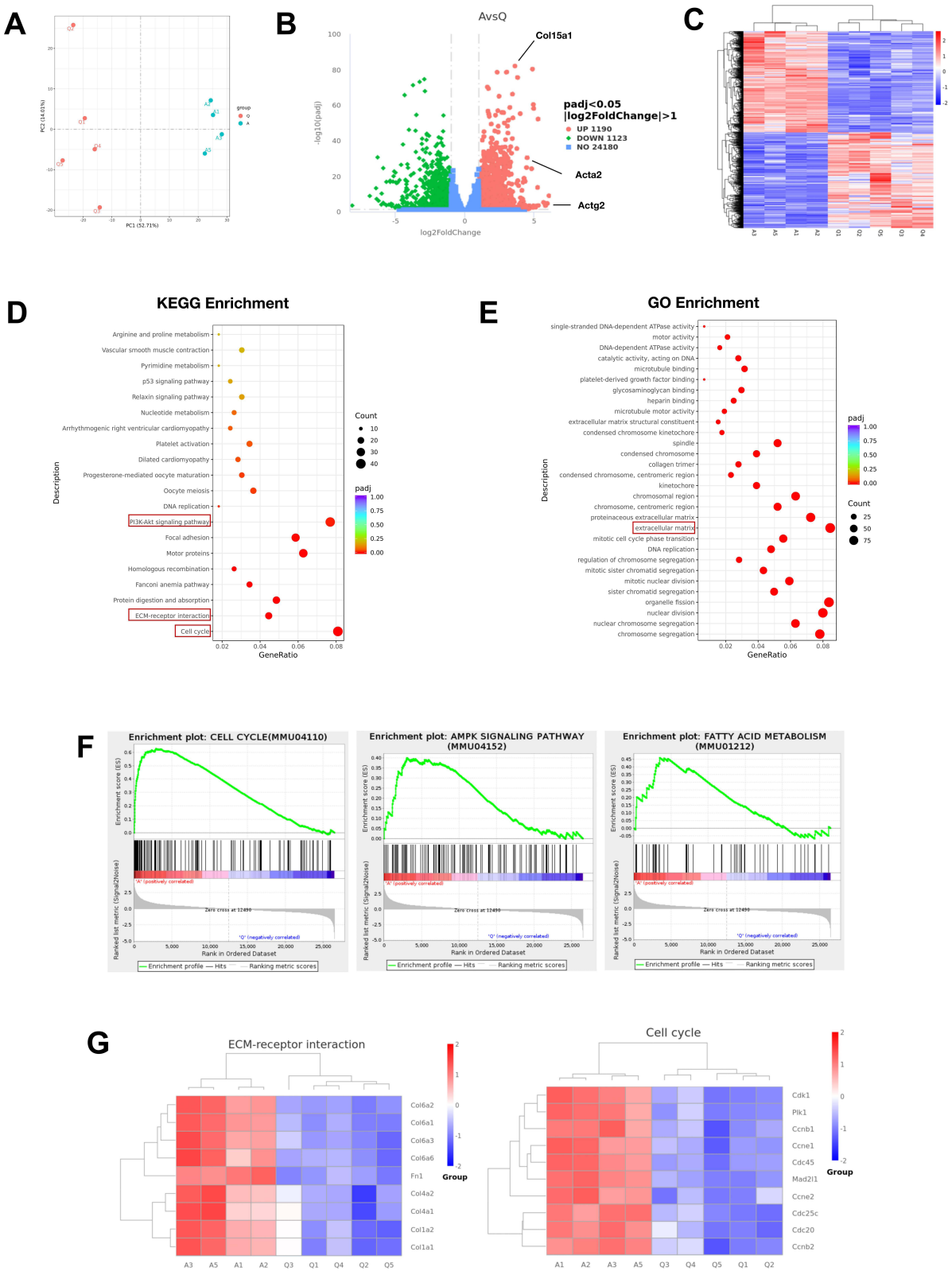

Figure 4 Altered mPSCs activated pattern was verified by transcriptomics analysis. (**A**) PCA analysis of mPSCs in caerulein-treated group and NS-treated group. The red dots represented the quiescent cells, and the blue dots represented the activated samples. The two major principal components explained 52.71% and 14.01% of the total variance, respectively. (**B**) The volcanic plot presenting the distribution of genes (|log2FoldChange| >1 and padj < 0.05). (**C**) The heatmap hierarchical clustering presenting the different distribution of two groups. (**D**) KEGG enrichment analysis of differentially expressed genes. (**E**) GO enrichment analysis of differentially expressed genes. (**F**) The GSEA figure of cell cycle, AMPK signaling pathway and fatty acid metabolism pathway. (**G**) Heatmap of ECM-receptor interaction and cell cycle - related gene expression profiles based on the RNA-seq data. n = 4–5 per group.

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(DEGs), KEGG and GO analyses were conducted. The DEGs enriched in pathways associated with increased stellate cell activation, survival, inflammation and energy metabolism in the caerulein-treated group compared to the NS-treated group, including the ECM-receptor interaction pathway, cell cycle, phosphatidylinositol 3-kinase–AKT (PI3K-Akt) signaling pathway and protein digestion and absorption, etc (Figure 4D). GO analysis revealed significant enrichment of the extracellular matrix pathway, indicating an abnormal increase in ECM disposition (Figure 4E). The GSEA results showed significant activation of the functions related to mPSCs activation, including the cell cycle, AMPK signaling pathway, and fatty acid metabolism (Figure 4F). Various DEGs in the ECM-receptor interaction pathway and cell cycle were shown in Figure 4G.

Collectively, these results demonstrated that short-term caerulein intraperitoneal injection is a novel and effective method for activating mPSCs in vivo, with caerulein-treated mPSCs exhibiting enhanced activation capacity.

Discussion

The activation of mPSCs is a key process in the abnormal remodeling of ECM in the pancreas and in the development of pathological fibrosis in CP. It is well established that mPSCs are the primary source of collagen and other ECM proteins during pancreatic fibrogenesis. Consequently, effective methods for isolating and culturing primary mPSCs in vitro are essential.

Various techniques for isolating stellate cells have been reported. Common methods include outgrowth from pancreatic tissue and density centrifugation, each offering distinct advantages. Cells that grow out from pancreatic tissues are in an activated state and are prone to aging after prolonged culture and passaging. Density centrifugation creates a density gradient in a centrifuge tube using a specific medium, allowing cells to be stratified and separated by gravity or centrifugal force. This method is frequently employed to isolate stellate cells in their quiescent phase; however, previously reported protocols primarily focused on isolating PSCs from rats.[5] Additionally, variations in the composition of density solutions, types of enzymes, enzyme concentrations, and digestion times have been observed across different studies. Furthermore, while mice are widely utilized as experimental models, a standardized method for isolating quiescent murine PSCs is lacking, which limits investigations into mouse PSCs. In our study, we proposed an efficient method for isolating abundant mouse PSCs with high activity and purity.

The activation of PSCs is the key to pancreatic fibrosis, with multiple molecular stimuli linked to PSC activation. Transforming growth factor-beta (TGF-β) is recognized as one of the most potent profibrogenic mediators.[13] Fibroblastic markers, including α-SMA and fibronectin, increased significantly in response to activation. Additionally, proinflammatory factors such as smoke, stress, reactive oxygen species, and platelet derived growth factor can enhance the fibrogenic activity of myofibroblasts.[14]

Previous studies on hepatic stellate cells indicate that the degree of activation increases with prolonged adherence.[15] However, there are few studies that illuminate the model of activated mPSCs established in vivo. Therefore, we investigated whether short-term intraperitoneal caerulein injection could serve as an effective method for establishing a mPSCs activation model, offering a novel approach for in vivo activation studies. Increased activation of PSCs is characterized by elevated levels of myofibroblast markers. Cell attachment promote activation. In contrast, our study allows for the simultaneous isolation of both quiescent and activated primary stellate cells directly from mouse. This approach circumvents the potential interference of in vitro experimental conditions on the metabolic phenotype of the cells, thereby providing a more physiologically relevant model for studying the activation of stellate cells.

Our results from RT-PCR and immunofluorescence staining proved an enhanced activation level following caerulein treatment. Additionally, the gene expression profile of mPSCs shifted to a highly activated and myofibroblast-like state, suggesting that caerulein injection is a potent method for establishing an activated model of mPSCs. RNA-seq analysis revealed pivotal signaling pathways by detecting variations in mRNA expression levels. In our results, specifically, the ECM-receptor interaction pathway was significantly upregulated in the KEGG analysis. The increased mRNA levels of collagen and fibronectin indicate the enhanced PSCs activated functions. The advantage of our modeling approach lies in the fact that it enables the concurrent acquisition of mPSCs in the quiescent and activated states, which is highly conducive to conducting cell function experiments.

Moreover, the proliferative capacity of activated PSCs relies on elevated biosynthetic functions, and cell proliferation requires an adequate energy supply. Previous studies have demonstrated that the activation of hepatic stellate cells is closely linked to

altered energy metabolism in both humans and mice.[16–18] Consistent with this, our results indicate that the enrichment of cell cycle, AMPK signaling pathway, and fatty acid metabolism reflects enhanced proliferation and metabolic capacity.

Additionally, the PI3K-Akt signaling pathway was significantly enriched in the KEGG analysis, representing a non-canonical pathway downstream of TGF-β. Previous literature has identified the PI3K-Akt signaling pathway as a key regulator of cell growth, survival, cell cycle progression, metabolism, and inflammation,[19] and has also been shown to promote the expression of TGF-β and PDGF, contributing to the activation of hepatic stellate cells (HSCs) and excessive extracellular matrix (ECM) production.[20,21]

The results above verified that the pretreatment with intraperitoneal caerulein injection serves as an effective method for establishing an ex-vivo model of mPSC activation. The activation of stellate cells exhibited increased activation levels, along with enhanced matrix-producing and proliferative phenotypes. The profiling of metabolic reprogramming and underlying mechanisms will be investigated in future studies.

Conclusion

Our work proposed a successful method for simultaneously isolating quiescent and activated PSCs from murine pancreas, providing a novel strategy for a deeper understanding of the mechanisms underlying mPSC activation. Short-term caerulein treatment serves as a reliable approach to activate PSCs in vivo. A major advantage of our model is its capacity to assess the altered characteristics of isolated mPSCs concurrently. Our study, in particular, avoid the potential confounding effects of in vitro cell attachment on the activation phenotype, thereby more accurately reflecting the true impact of the research target on mPSCs activation.

Ethical Statement

Animal experiments were approved by the Institutional Animal Care and Use Committee of Capital Medical University (Approval number: AEEI-2024-005; Date: 11 January 2024) and followed the ARRIVE guidelines.[22] All procedures were conducted in accordance with the *National Institutes of Health's Guide for the Care and Use of Laboratory Animals*.

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Disclosure

The authors report no conflicts of interest in this work.

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