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ORIGINAL RESEARCH

Bone Marrow Mesenchymal Stem Cells Can Prevent Pancreatic Fibrosis in Mice with Chronic Pancreatitis by Inhibiting the Activation of Pancreatic Stellate Cells

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Background: Chronic pancreatitis (CP) is a progressive fibroinflammatory disorder primarily driven by a complex interplay of environmental and genetic risk factors. Fibrosis, mediated by activated pancreatic stellate cells (PSCs), represents a key pathological feature of CP. Bone marrow mesenchymal stem cells (BMSCs) are known for their anti-fibrotic and anti-inflammatory properties; however, their role in pancreatic fibrosis remains inadequately understood.

Aim of the Study: This study aims to investigate the effects of BMSCs on CP and elucidate the underlying mechanisms.

Methods: To evaluate the effects of BMSCs on pancreatic tissue alterations in CP, a mouse CP model was established using Ceruletide, followed by tail vein injection of BMSCs. Histological assessments of pancreatic injury were performed using hematoxylin and eosin (H&E) staining and Masson's trichrome staining. The role of BMSCs in PSC activation and function was investigated through co-culture experiments with PSCs. Furthermore, to elucidate the underlying mechanisms by which BMSCs influence PSCs, transcriptomic analysis of PSCs was performed to identify key molecules and signaling pathways.

Results: In vivo, BMSCs mitigated pathological changes and collagen deposition while suppressing PSC activation in CP mice. In vitro, BMSCs inhibited PSC activation and function, as evidenced by reduced collagen secretion, decreased proliferation, and increased apoptosis. RNA sequencing identified the aryl hydrocarbon receptor (AhR) and peroxisome proliferator-activated receptor gamma (PPARγ) as potential key mediators. Notably, AhR antagonism reversed the inhibitory effects of BMSCs on PSCs and attenuated the BMSC-induced upregulation of PPARγ in PSCs.

Conclusion: Transplantation of BMSCs suppresses PSCs activation and attenuates the pancreatic fibrosis in CP. This process may be mediated by the activation of AhR and PPARγ in PSC.

Keywords: chronic pancreatitis, pancreatic stellate cells, pancreatic fibrosis, α-smooth muscle actin, bone marrow mesenchymal stem cells

Introduction

Chronic pancreatitis (CP) is a chronic, fibroinflammatory syndrome with a prevalence of approximately 50 cases per 100,000 population. It is characterized by abdominal pain and pancreatic exocrine-endocrine dysfunction, which significantly impairs the quality of life for patients. Additionally, there is an increased risk of pancreatic cancer associated with CP.[1,2] Developing preventive and therapeutic strategies for CP is, therefore, of paramount importance.

The primary pathological changes in CP are acinar cell injury, inflammation, and fibrosis.[2] Activated pancreatic stellate cells (PSCs) play a crucial role in fibrogenesis.[3] In normal physiological conditions, PSCs maintain a quiescent

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phenotype. Once stimulation by various pathogenic factors, quiescent PSCs become activated and differentiate into myofibroblast-like cells.[4] The expression of α-smooth muscle actin (α-SMA), also known as alpha actin 2 (Acta2), is a marker of PSC activation.[5] Activated PSCs exhibit increased proliferation and extracellular matrix (ECM) production, driving fibrosis progression in CP.[6] Reducing PSC activation and function may, therefore, offer a viable approach to mitigating fibrosis.

Mesenchymal stem cells (MSCs) constitute a heterogeneous subpopulation of stromal stem cells, which are characterized by multilineage differentiation potential and immunomodulatory capabilities. They can be derived from numerous tissues and play a pivotal role in tissue homeostasis and repair. Recent studies have demonstrated that MSCs exert a significant inhibitory effect on the progression of fibrosis in multiple organs, including the liver,[7] lungs,[8] kidneys[9] and peritoneum.[10] In liver fibrosis, MSCs inhibit hepatic stellate cell (HSC) activation via various mechanisms,[11] including the secretion of factors like milk fat globule-EGF factor 8 (MFGE8) and insulin-like growth factor binding protein-3 (IGFBP-3).[12–14]

Whether MSCs exert similar inhibitory effects on PSCs remains unclear. In the pancreas, current evidence is limited to a rat model study showing that MSCs attenuate CP-related fibrosis by inhibiting the PI3K/AKT/mTOR and TGF-β/SMAD signaling pathway.[15–17] Other studies have reported that MSCs can downregulate the expression of TGF-β, platelet-derived growth factor (PDGF), intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1 (VCAM-1) in pancreatic tissue, suggesting that these may be potential targets for suppressing PSC activation and fibrosis.[18] However, the precise mechanisms remain to be elucidated.

Bone marrow mesenchymal stem cells (BMSCs) are the earliest discovered and most abundant subset of MSCs.[19,20] BMSCs are easily isolated from bone marrow and hold great potential for clinical application;[21] therefore, BMSCs were selected to investigate the therapeutic effects of MSCs on PSCs and pancreatic fibrosis in CP, and to explore the potential mechanisms involved.

Methods and Materials

Cell Culture

Mouse PSCs were isolated from male C57BL/6N mice, aged 6 weeks with a body weight ranging from 20 to 22 grams. Primary cells were cultured in DMEM/F12 medium (Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% penicillin/streptomycin antibiotics (P/S, Biological Industries), as described in our previous studies.[22,23] Cells from passages 2–3 were utilized for the in vitro experiments.

Mouse BMSCs were isolated from male C57BL/6N mice, aged 3 weeks with a body weight ranging from 10 to 12 grams.[24] Primary cells were cultured in MEMα medium (Gibco) with 10% FBS and 1% P/S. Cells from passages 3 were utilized for experiments.

CP Model Induction and BMSCs Transplantation

All mice used in the experiments were procured from Charles River Laboratories. The mice used in the vivo experiments were male C57BL/6N mice, aged 8 weeks with a body weight ranging from 22 to 24 g, as described in our previous studies.[25] All animal experiments were conducted in strict adherence to the ARRIVE guidelines to ensure the welfare of the animals.[26]

The mice were randomly divided into control group, CP model group (CP group), and transplantation of BMSCs group (CP+BMSCs-tvi group). Mice in CP group and CP+BMSCs-tvi group were injected with Ceruletide (Target Mol) via intraperitoneal injection at a concentration of 50μg/kg for 6 times per day, and three days per week for 4 consecutive weeks. Concurrently, mice in control group received an equivalent volume of physiological saline.[27,28]

Before the first dose of Ceruletide each week, the mice in CP+BMSCs-tvi group were injected 1.5×10^5 BMSCs in 250μL of Phosphate Buffered Saline (PBS) into the tail vein. Mice in other group were injected with an equal volume of PBS as a vehicle control.[29,30]

Co-Culture Experiment

PSCs were seeded at a density of 80,000 cells per well in a six-well plate, with each well adding 2.6 mL of DMEM/F12 medium with 10% FBS and 1% P/S. For the co-culture group, an equivalent number of BMSCs were seeded in a transwell chamber (Corning 3412) to stimulate the PSCs in the lower well, with each chamber adding 1.4 mL of DMEM/F12 medium with 10% FBS and 1% P/S. 30% of the conditioned medium from BMSCs (BMSC-CM) was added to each wells in the BMSC-CM group. For the BMSC-CM group and control group, the medium was supplemented to a final volume of 4 mL, with the blank transwell inserting.[14,31,32]

Cell Proliferation

Cell Counting Kit-8 Assay (CCK-8, Lablead) was used for evaluating cell proliferation and viability. PSCs were seeded in 96-well plates (2000 cells/well). Cell proliferation and viability were assessed via measuring the absorbance at 450 nm by microplate reader (Thermos Fisher).

Cell Apoptosis

Annexin V-PE/7-AAD Apoptosis Detection Kit (Vazyme) was used for evaluating cell apoptosis. About 5×10^5 cells were collected and stained for analysis via flow cytometry. The subsequent data were analyzed by FlowJo software. The total number of apoptotic cells included the sum of early and late apoptotic cells.

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

RNA was isolated from PSCs and pancreatic tissues by TRIzol reagent (Invitrogen). Reverse transcription was performed using the PrimeScript RT Master Mix (Takara). Quantitative PCR (qPCR) was executed by ABI 7500 platform (Applied Biosystems) with SYBR Green Master Mix (Vazyme). The results were analyzed using the Livak method. Primers listed in Supplementary Table 1.

Western Blotting Analysis

Proteins were extracted from mouse (PSCs) and pancreatic tissues using RIPA buffer (Solarbio) supplemented with protease and phosphatase inhibitors. Protein concentrations were determined by the BCA protein assay kit (Thermos Fisher). Following electrophoresis, protein samples were transferred to PVDF membranes. The membranes were then incubated with the primary antibodies overnight at 4°C, followed by incubation with fluorescent secondary antibodies for 1 hour. The Odyssey fluorescence scanner (LI-COR) was used for detection. Details of the antibodies used are provided in Supplementary Table 2.

Collagen Gel Contraction

Based on previous literature,[33] suspensions with 20,000 PSCs were mixed with rat tail collagen type 1 (Corning) on ice and then added to 24-well plates. After incubation at 37°C for 1 hour to allow collagen solidify, 500 μL of culture medium was added to suspend the gels. The contractility of PSCs was assessed by evaluating the change in gel size over time after 24 hours.

Histology

Pancreatic tissues were fixed in a 4% paraformaldehyde solution for histological preservation. The extent of pancreatic fibrosis was quantified using hematoxylin and eosin (H&E) for general tissue morphology, and Masson's trichrome for collagen deposition assessment. These staining procedures were conducted by Servicebio technology.

RNA Sequencing

Total RNA was extracted from PSCs using TRIzol reagent. The sequencing process and analysis were conducted by Novogene corporation.

Statistical Analysis

Data are presented as the mean ± standard deviation (SD) and were analyzed by SPSS (version 27.0, Chicago, IL, USA). Student's *t*-tests or one-way analysis of variance (ANOVA) were utilized to assess differences between each groups. P<0.05 was considered statistically significant. Graphs and visual illustrations were generated using GraphPad Prism (version 10.1, San Diego, CA, USA) and Novo-Magic tools (https://magic-plus.novogene.com/#/).

Results

Transplantation of BMSCs Alleviates Pancreatic Fibrosis in CP Mice

Detailed procedures for CP model induction and BMSC treatment are described in the Methods section (Figure 1A). After four weeks of Ceruletide treatment, the CP group exhibited a significant reduction in body weight compared to the control group (p<0.0001). While mice in the CP + BMSCs-tvi group also experienced weight loss, the decrease was less pronounced than in the CP group (P>0.05) (Figure 1B). Similarly, the pancreatic weight-to-body weight ratio (P/W) was significantly lower in the CP group compared to the control group (p<0.0001). However, this ratio was notably improved in the CP + BMSCs-tvi group relative to the CP group (P<0.05) (Figure 1C). Furthermore, mice in the CP group displayed marked histological alterations in pancreatic tissue, including acinar atrophy, inflammatory infiltration, fibrosis, and collagen deposition. In contrast, these pathological changes were significantly attenuated in the CP + BMSCs-tvi group (Figure 1D).

The expression of fibrosis-related genes, including collagen type I (*Col1*), collagen type III (*Col3*), and fibronectin 1 (*Fn1*), was significantly elevated in the CP group compared to the control group (*Col1* p<0.001; *Col3* p<0.0001; *Fn1* p<0.05). However, their expression levels were substantially reduced in the CP + BMSCs-tvi group relative to the CP group (*Col1* p<0.05; *Col3* p>0.05; *Fn1* p<0.05) (Figure 1E). Western blot analysis corroborated these findings, revealing upregulated expression of α-SMA (p<0.01) and COL1 (p<0.001) in the CP group. Notably, these protein levels were significantly lower in the CP + BMSCs-tvi group (α-SMA p<0.05; COL1 p<0.05) (Figure 1F).

In summary, these findings suggest that transplantation of BMSCs can alleviate Ceruletide-induced pancreatic fibrosis in vivo.

BMSCs Inhibit the Activation and Function of PSCs in vitro

Given that BMSC transplantation reduced α-SMA levels in the pancreatic tissues of CP mice, we proposed that the antifibrotic effects of BMSCs are mediated through the inhibition of PSC activation and functionality.

In vitro co-culture experiments revealed that, compared to PSCs cultured alone in the control group, PSCs co-cultured with BMSCs exhibited significantly reduced proliferative capacity after 72 hours (p<0.01) (Figure 2A). Flow cytometry further demonstrated a substantial increase in the apoptosis rate of PSCs in the co-culture group compared to the control group (p<0.05) (Figure 2B). Western blot analysis indicated downregulation of the anti-apoptotic protein B-cell lymphoma-2 (BCL-2) in the co-culture group (p<0.05), whereas the pro-apoptotic protein Bcl-2 Associated X Protein (BAX) showed no significant change (p>0.05) (Figure 2C).

Subsequently, we assessed the changes in activation markers of PSCs. Both Western blot and qPCR results demonstrated that the protein and mRNA level of α-SMA in PSCs in the co-culture group was lower compared to the control group (*Acta2* p<0.001; α-SMA p<0.05). The protein and mRNA level of COL1 was also significantly down-regulated in the co-culture group (*Col1* p<0.05; COL1 p<0.05). Additionally, the mRNA levels of *Col3, Fn1* and several enzymes that involved in ECM metabolism were measured, including matrix metalloproteinase 13 (*Mmp13*), metallo-peptidase inhibitor 1 (*Timp1*), metalloproteinase 2 (*Mmp2*) and metallopeptidase inhibitor 2 (*Timp2*). In the co-culture group, the mRNA levels of *Col3* (p<0.05) and *Fn1* (p<0.05) were significantly downregulated, while the mRNA level of *Mmp13* was upregulated (p<0.001), and the mRNA levels of other genes did not show significant changes (Figure 2D and E). Finally, a collagen gel contraction assay revealed that PSCs in the co-culture group exhibited diminished contractility, as evidenced by a larger gel area compared to the control group (p<0.01) (Figure 2F).

In conclusion, these findings indicate that BMSCs effectively inhibit PSC activation, proliferation, and collagen production while promoting PSC apoptosis in vitro.

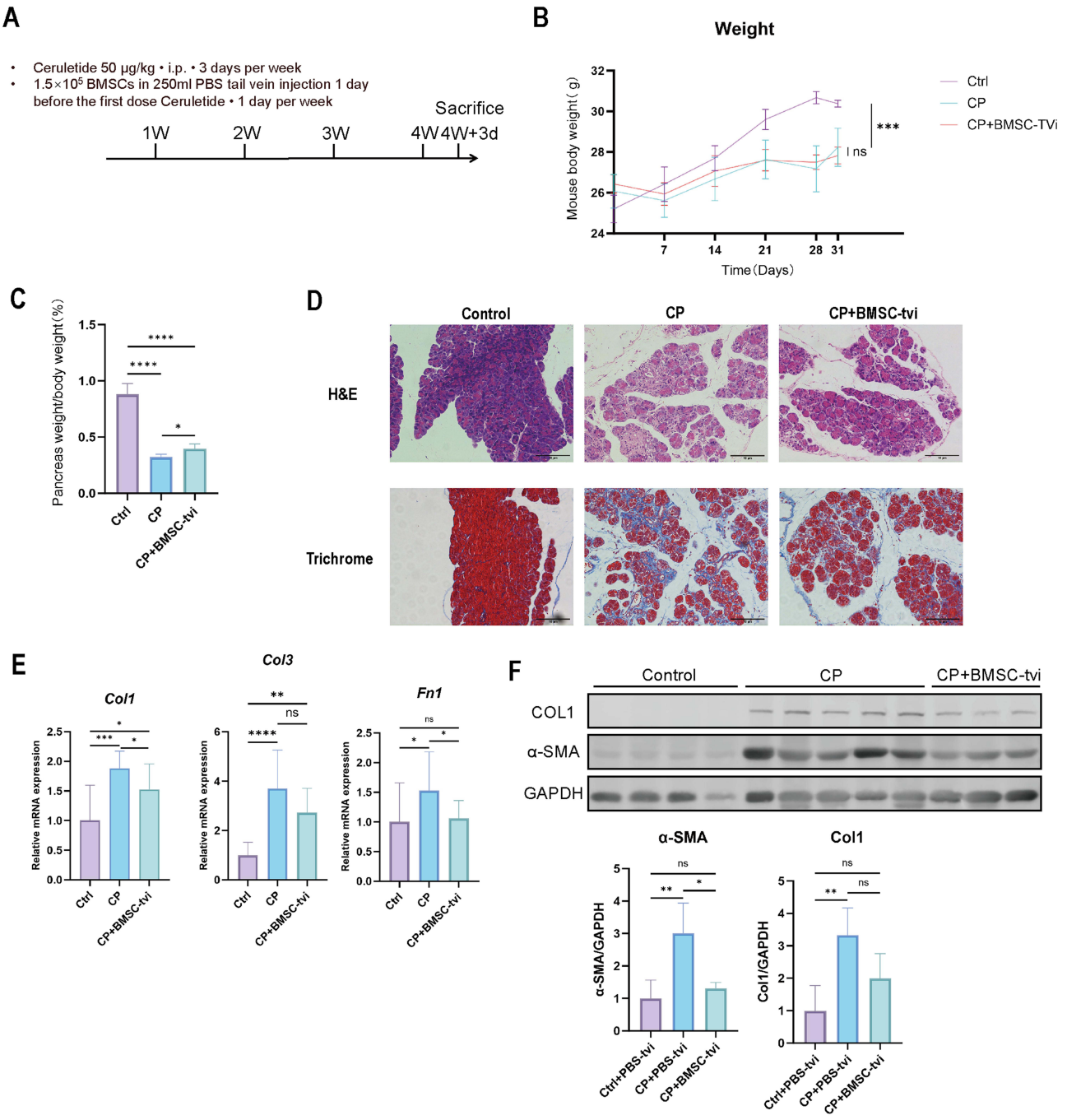

Figure 1 Transplantation of BMSCs alleviates pancreatic fibrosis in CP mice. (**A**) Flow chart of animal experiment (i.p: intraperitoneal injection). (**B**) Mice Body Weight changes during CP Induction. (**C**) P/W after mice sacrifice. (**D**) H&E and Masson trichrome staining of the pancreatic tissue. (**E**) mRNA level of fibrosis-related genes. (**F**) Protein level of α-SMA and COL1 in pancreatic tissue. (Statistical significance: ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

Transcriptomic Characteristics of PSCs Co-Cultured with BMSCs

To elucidate the mechanisms by which BMSCs influence PSC activation and function, RNA sequencing was performed on PSCs from the co-culture and control groups. Principal component analysis (PCA) revealed distinct transcriptomic profiles between the two groups (Figure 3A). Differentially expressed genes (DEGs) were identified using the criteria of $|\log_2(\text{FoldChange})| > 1$ and adjusted p-value (padj) < 0.05. In total, 86 genes were upregulated, and 64 genes were downregulated in the co-culture group compared to the control group (Figure 3B).

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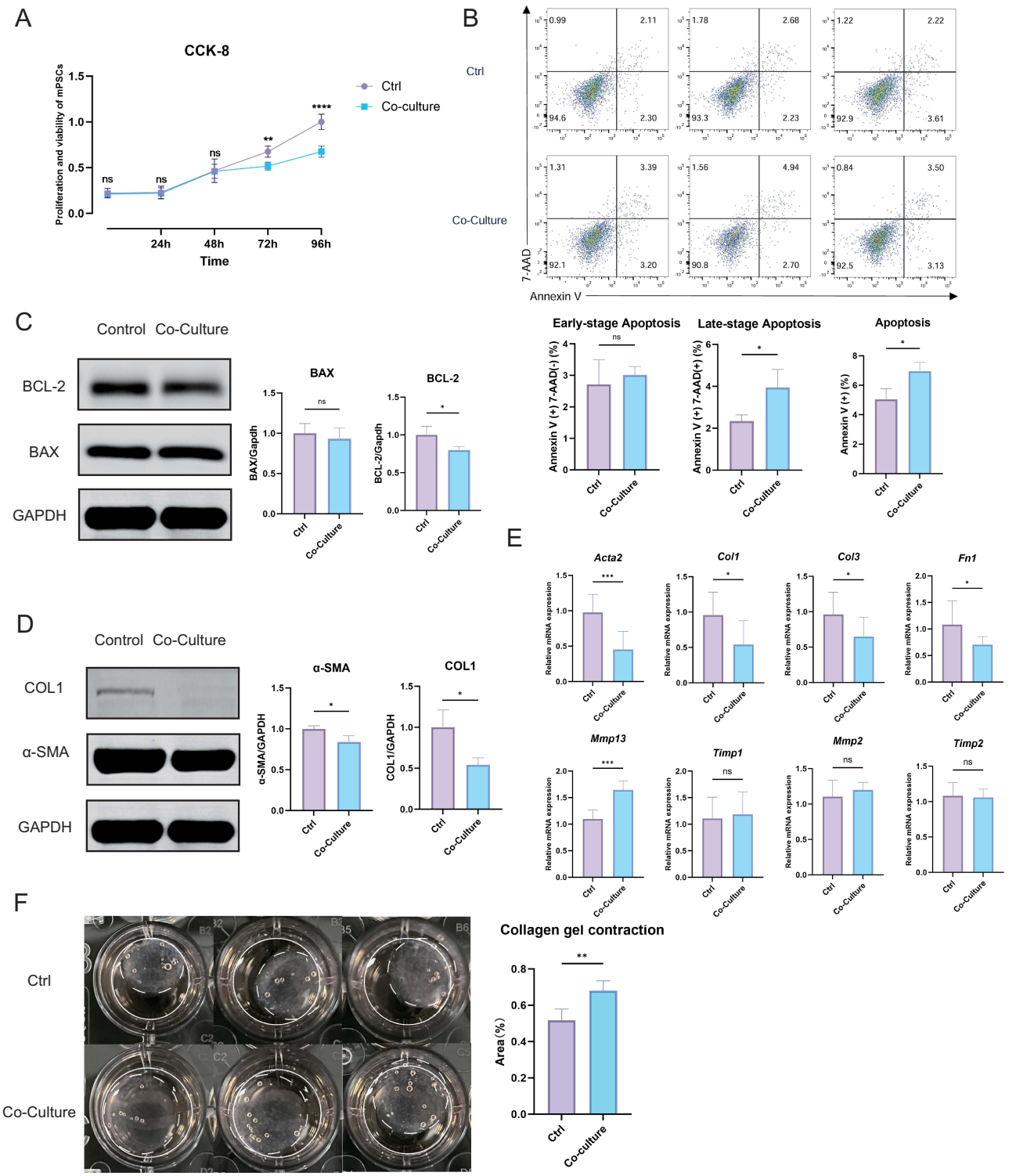

Figure 2 BMSCs inhibit the activation and function of PSCs in vitro. (**A**) Relative cell proliferation and viability of mPSCs determined by CCK-8 assays. (**B**) Apoptotic rates of mPSCs detected by flow cytometry. (**C**) The protein levels of BCL-2 and BAX in mPSCs. (**D**) The protein levels of α-SMA and COL1 in mPSCs. (**E**) mRNA levels of α-SMA and fibrosis-related genes in mPSCs. (**F**) Changes in collagen contractility were observed after Co-culture. (Statistical significance: ns, not significant, *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

Gene Ontology (GO) enrichment analysis was conducted to explore the biological significance of these DEGs. In the "cellular component" category, the top enriched terms were related to extracellular matrix metabolism, while in the "molecular function" category, several terms associated with receptor activity ranked highly (Figure 3C). Given this, the

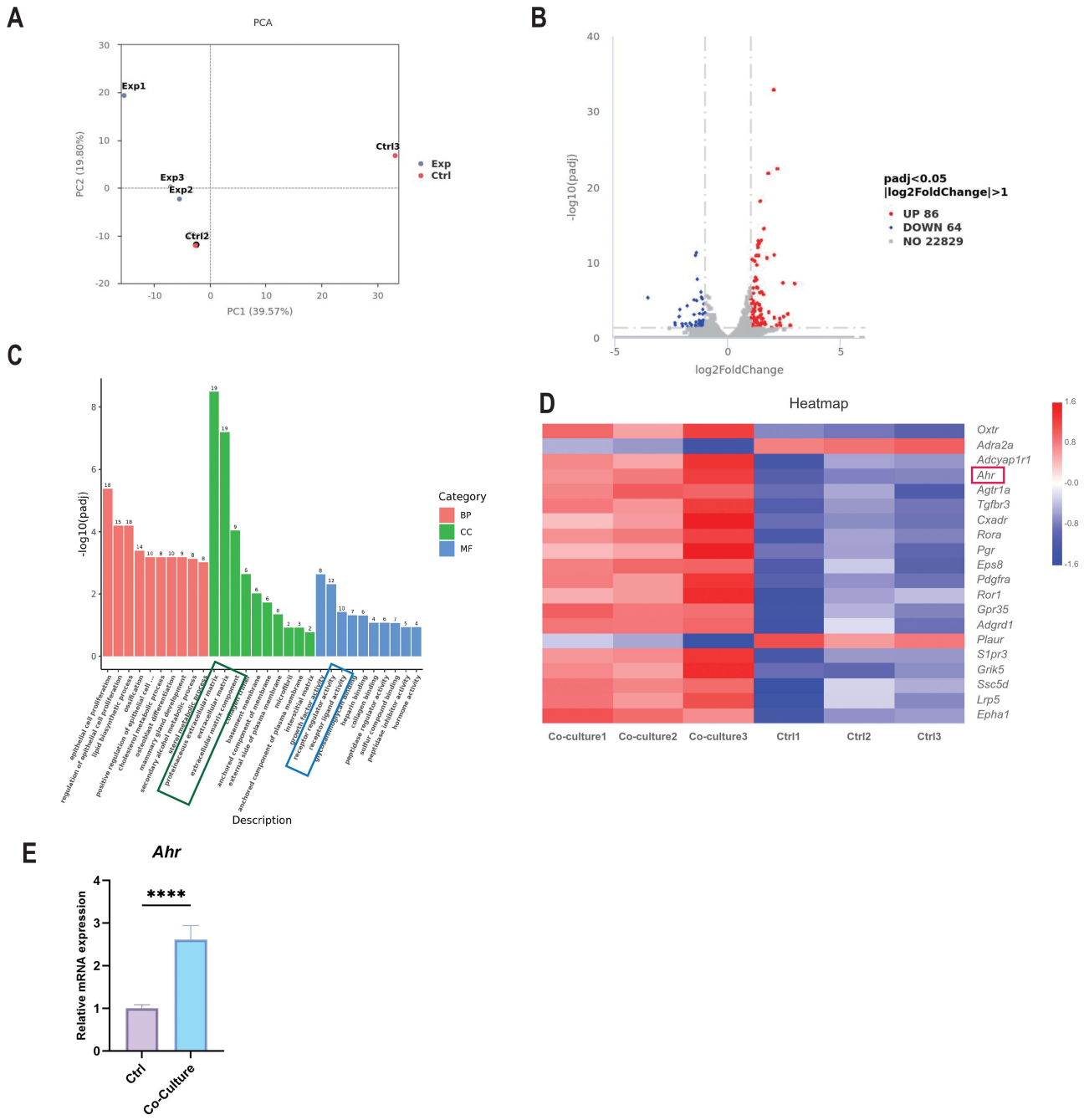

Figure 3 Transcriptomic characteristics of PSCs co-cultured with BMSCs. (**A**) PCA illustrates the variance of all genes expressed in each sample. (**B**) Volcano plot of DEGs in both co-culture group and control group (log2|FoldChange|>1 and padj<0.05). (**C**) The top 10 GO terms in biological process, cellular component, and molecular function of DEGs. (**D**) The top 20 differentially expressed receptors are shown in a heatmap. (**E**) mRNA levels of AhR in mPSCs. (Statistical significance: ****P < 0.0001).

top 20 differentially expressed receptors were visualized in a heatmap (Figure 3D). Among these, the aryl hydrocarbon receptor (AhR), ranked third, has been implicated in HSCs activation and LF.[34] Subsequent qRT-PCR analysis confirmed a significant upregulation of AhR mRNA expression in the co-culture group compared to the control group (p<0.0001) (Figure 3E). These findings suggest that AhR may play a critical role in mediating the antifibrotic effects of BMSCs on PSCs.

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BMSCs Inhibit the Activation and Function of PSCs Through the Activation of AhR

Based on the observed upregulation of AhR in PSCs co-cultured with BMSCs, we further investigated its role in modulating PSC activation and functionality.

Treatment of PSCs with varying concentrations and durations of the AhR agonist ITE significantly inhibited PSC proliferation, as shown by CCK-8 assays (Figure 4A). Western blot analysis revealed that ITE treatment (1 μM, 48 hours) markedly reduced the expression of α-SMA (Figure 4B). Additionally, qRT-PCR confirmed that ITE significantly downregulated the mRNA expression of *Acta2, Col1*, and *Col3* in PSCs (Figure 4C).

To validate the role of AhR, PSCs were treated with BMSC-CM in the presence of the AhR antagonist PDM2. BMSC-CM significantly inhibited the viability of PSCs at 96 hours, and the effect was reversed by the application of PDM2 (Figure 4D). Western blot analysis showed that BMSC-CM significantly reduced the expression of the anti-apoptotic protein BCL-2 ($p<0.05$), an effect attenuated by PDM2 (500nM, $p<0.05$). No significant differences were observed in BAX expression across groups (Figure 4E). Flow cytometry further demonstrated that BMSC-CM increased PSC apoptosis ($p<0.0001$), while this effect was partially reversed by PDM2 (500 nM, $p<0.01$) (Figure 4F). Moreover, BMSC-CM significantly downregulated the protein levels of α-SMA ($p<0.01$) and COL1 ($p<0.05$), both of which were restored upon PDM2 treatment (Figure 4G). Similar trends were observed for the mRNA expression of *Acta2, Col1, Col3*, and *Fn1* (Figure 4H).

In summary, these findings indicate that BMSCs inhibit the activation and function of PSCs through the activation of AhR.

AhR May Suppress the Activation and Function of PSCs via the Activation of the PPAR Pathway

To explore the signaling pathways underlying the functional changes in PSCs induced by BMSCs, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed on DEGs. The results highlighted the PPAR signaling pathway as the most significantly enriched pathway (Figure 5A). Among PPAR family members, PPARγ has been strongly associated with antifibrotic effects. Further analysis revealed that mRNA expression of *Pparγ* was significantly upregulated in PSCs co-cultured with BMSCs compared to the control group ($p<0.0001$) (Figure 5B).

To confirm the interplay between AhR and PPARγ, we measured PPARγ expression in PSCs treated with BMSC-CM in the presence of PDM2. The results showed a marked increase in protein and mRNA levels of PPARγ in the BMSC-CM group, which was significantly attenuated by PDM2 treatment (Figure 5C and D).

These findings suggest that the antifibrotic effects of BMSCs on PSCs may be mediated, at least in part, by upregulation PPARγ.

Discussion

An increasing number of studies have found that MSCs exert both preventive and therapeutic effects in the development of fibrotic diseases across various organs.[7–10,35] The majority research on the anti-fibrotic effects of MSCs is concentrated on hepatic and pulmonary fibrosis.[36,37] However, few studies have demonstrated the ability of MSC transplantation to alleviate CP and pancreatic fibrosis in vivo. Notably, these investigations were primarily conducted in rat models and lacked exploration of the underlying mechanisms.[15,16,18] In this study, we observed that the transplantation of BMSC prevented pancreatic fibrosis during the progression of CP in mice, primarily through inhibiting PSC activation and function potentially by the activation of AhR and PPARγ.

Body weight changes and the P/W ratio are important indicators for assessing the severity of CP.[22,38,39] In our study, the change in mice body weight was not significant, but the P/W ratio showed a marked improvement following transplantation of BMSCs in CP mice. In addition, the transplantation of BMSCs improved the pathological features and reduced collagen deposition, accompanied by suppressed PSCs activation, consistent with previous findings in rat CP models.[16,18] These findings indicate that transplantation of BMSCs alleviates CP and pancreatic fibrosis in mice.

In studies on LF, MSCs have been shown to inhibit fibrosis by suppressing the activation and function of HSCs, a process mediated by various factors secreted by MSCs.[21,40,41] Considering that PSCs play a similar role in pancreatic

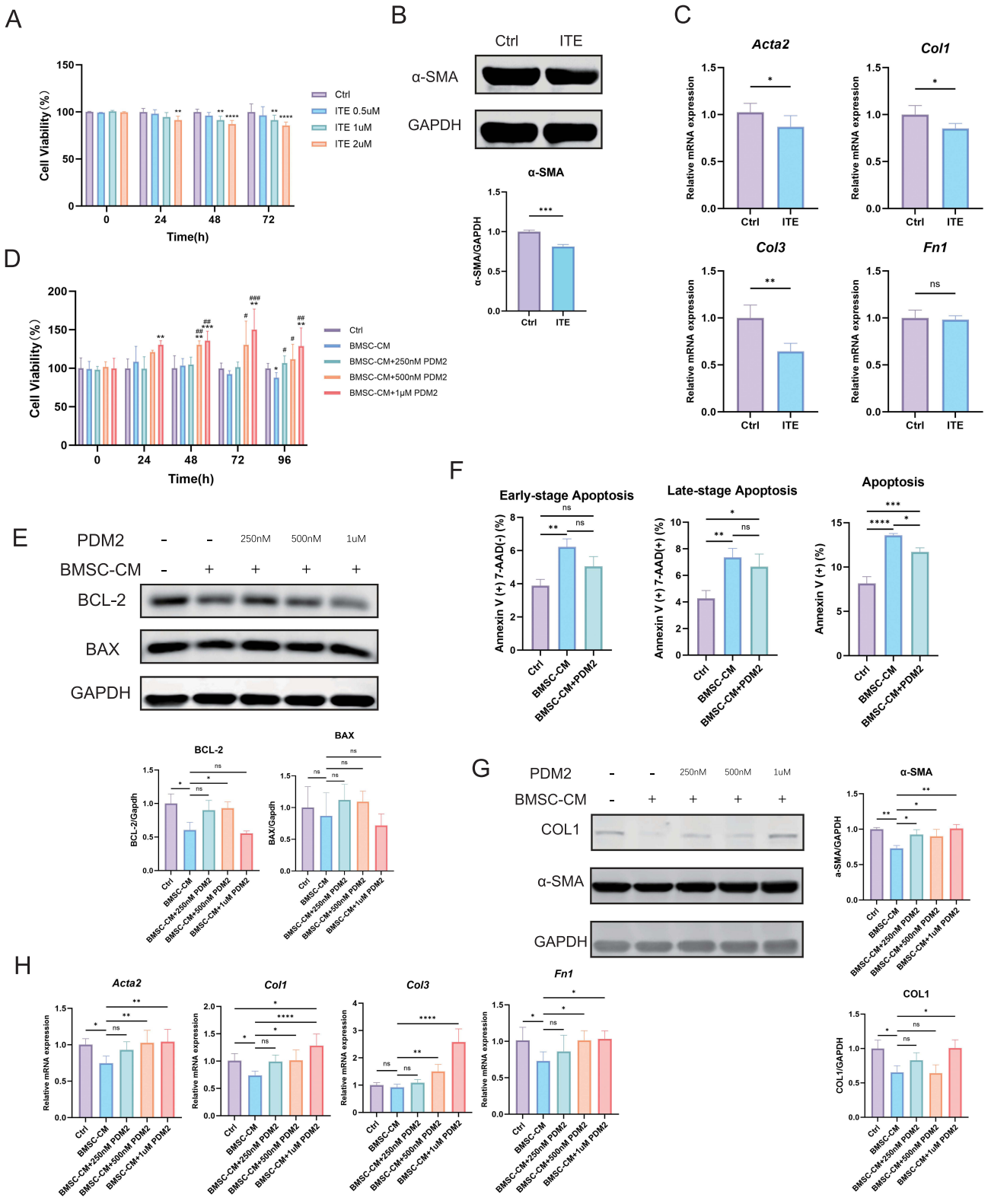

Figure 4 BMSCs inhibit the activation and function of PSCs through the activation of AhR. (**A**) Cell viability of mPSCs stimulated with ITE determined by CCK-8 assays. (**B**) The protein levels of α-SMA in mPSCs stimulated with ITE. (**C**) mRNA levels of α-SMA and fibrosis-related genes in mPSCs stimulated with ITE. (**D**) Relative cell proliferation and viability of mPSCs determined by CCK-8 assays. (**E**) The protein levels of BCL-2 and BAX in mPSCs. ("+" indicates treatment with BMSC-CM, while "–" indicates no treatment with PDM2 or BMSC-CM). (**F**) Apoptotic rates of mPSCs detected by flow cytometry. (**G**) The protein levels of α-SMA and COL1 in mPSCs. ("+" indicates treatment with BMSC-CM, while "–" indicates no treatment with PDM2 or BMSC-CM.) (**H**) mRNA levels of α-SMA and fibrosis-related genes in mPSCs. (Statistical significance: ns, not significant; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; in (**D**) *indicates comparison with the control group; #indicates comparison with the BMSC-CM group).

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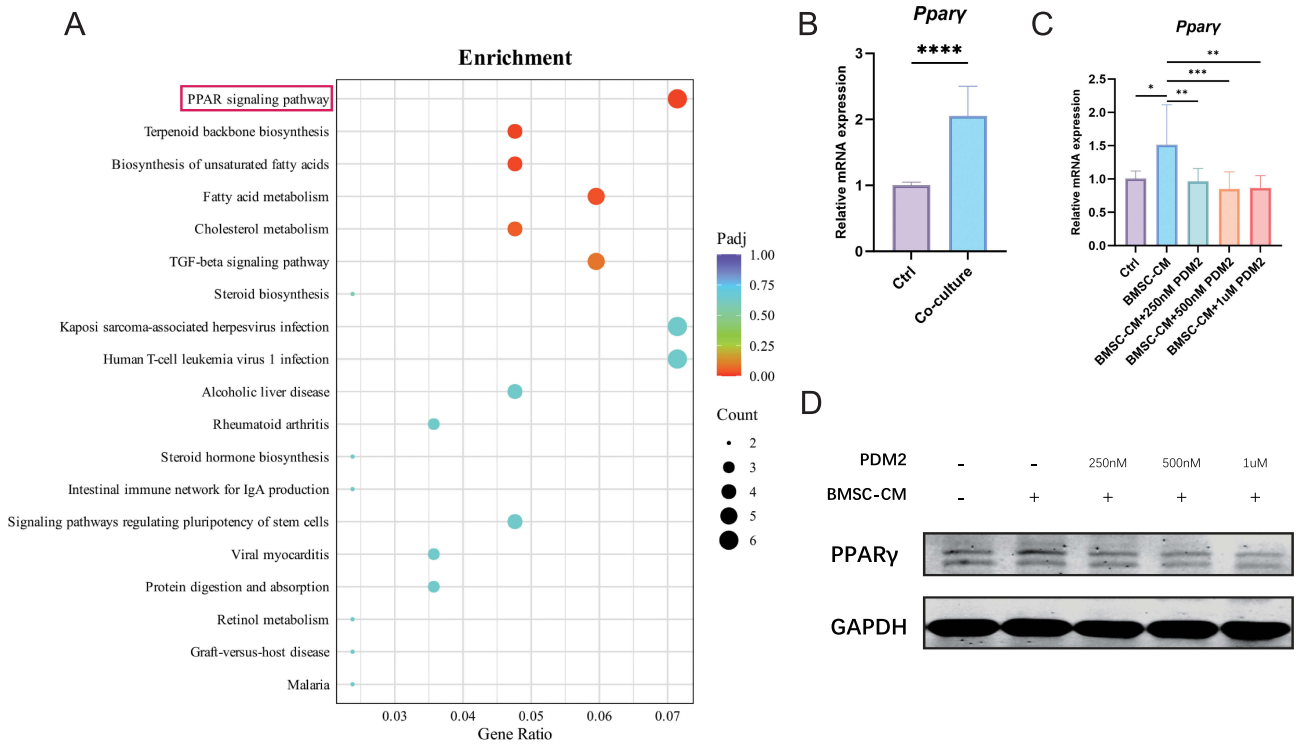

Figure 5 AhR may suppress the activation and function of PSCs via the activation of the PPAR pathway. (**A**) KEGG enrichment analysis of DEGs. (**B** and **C**) The mRNA levels of PPARγ in mPSCs. (**D**) The protein levels of PPARγ in mPSCs. ("+" indicates treatment with BMSC-CM, while "–" indicates no treatment with PDM2 or BMSC-CM). (Statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

fibrosis, and transplantation of BMSCs suppressed the activation of PSCs in vivo, we investigated the effects of BMSCs on PSCs in vitro. PSCs spontaneously activate during the vitro culture, so we did not employ any pharmacological agents to induce activation of PSCs.[42] Our study discovered that BMSCs suppress the activation of PSCs in vitro, while simultaneously inhibiting the proliferation, enhancing the apoptosis of PSCs, and reducing the production of ECM components. These findings have been rarely reported in previous studies.

RNA sequencing was performed on PSCs from both the co-culture and control groups to explore the mechanisms underlying PSC activation and functional changes. The GO analysis revealed that the molecular functions of the DEG are associated with receptor activity. Therefore, we conducted a screening of differentially expressed receptors. We found that among the top 5 receptors, aside from AhR, the remaining receptors have been seldom associated with fibrotic diseases in previous studies. AhR, an ligand-activated transcription factor, is one of the many crucial sensors involved in detecting changes in the cellular environment.[43] The role of AhR in fibrotic diseases has previously been a point of debate. Early studies suggested that 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) could promote liver fibrosis through the activation of AhR.[44,45] However, subsequent research indicated that high doses of TCDD possess hepatotoxicity and disrupts AhR signaling pathways. Furthermore, the absence of AhR in bone marrow cells has been shown to exacerbate diet-induced liver fibrosis.[46] Other studies have found that the non-toxic AhR agonist ITE alleviates liver fibrosis by inhibiting the activation of HSCs.[34] We discovered that PDM2 can reverse the inhibition of activation and collagen production, as well as the increase in apoptosis rate in PSCs induced by BMSC-CM. Therefore, we propose that BMSCs suppress the activation and functional changes of PSCs by activating the AhR in vitro.

KEGG analysis revealed that the DEGs were predominantly enriched in the PPAR signaling pathway. PPARs, including PPARα, PPARβ/δ, and PPARγ, are members of the nuclear receptor superfamily and function as ligand-activated transcription factors. They play a crucial role in regulating glucose and lipid metabolism.[47] In addition, the activation of PPARγ is considered a strong correlation with the inhibition of fibrosis.[47,48] In chronic kidney disease (CKD), it has been discovered that the crosstalk between AhR and PPARγ contributes to the progression of renal fibrosis. This process may involve the interaction with heat shock protein-90.[49] Moreover, activation of PPARγ in HSCs has been

shown to significantly suppress their activation and alleviate LF by inhibiting HSC proliferation and promoting HSC apoptosis.[14,50] Similarly, in PSCs, the activation of PPARγ is negatively correlated with PSC activation.[51,52] In our study, treatment with BMSCs significantly activated PPARγ in PSCs. This effect was abolished upon the application of PDM2, suggesting that the impact of BMSCs on PSCs may be mediated through PPARγ activation. Although research on the progression of CKD[49] and the treatment of neuroblastoma[53] has identified crosstalk between AhR and PPARγ,[54] the existence of similar interactions in PSCs needs further exploration.

There are certain limitations in this study. In studies of MSC-based therapies for various diseases, transplanted MSCs have been shown to accumulate primarily in highly vascularized organs such as the lungs and liver,[14,55] whereas their accumulation in less perfused organs like the pancreas is limited or absent.[55,56] In the present study, we found that transplantation of BMSCs alleviated pancreatic fibrosis in CP mice, and the inhibitory effect of BMSCs on PSCs was mediated through indirect secretory mechanisms in vitro. Previous studies have indicated that, in addition to exerting direct effects at the site of injury, MSCs can also act indirectly by secreting soluble factors, extracellular vesicles, miRNAs, and transferring mitochondria.[57–59] Our findings further support this concept, suggesting that MSCs can exert therapeutic effects through the secretion of "key molecules". However, the specific "key molecules" responsible for MSC-mediated inhibition of PSC activation and pancreatic fibrosis remain unclear and warrant further investigation.

Conclusion

The present study appeared that BMSCs transplantation can alleviate severity of CP and pancreatic fibrosis. This process is partly mediated through the suppression of PSC activation and function possibly by the activation of AhR and PPARγ. These findings provide a reference for the mechanisms and applications of BMSCs in the prevention of CP.

Ethical Approval

This study was approved by the Animal Experiments and Experimental Animal Welfare Committee of Capital Medical University (AEEI-2024-005).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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