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Effect of the IκBα mutant gene delivery to mesenchymal stem cells on rat chronic pancreatitis

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ABSTRACT. This study aimed to investigate the effect of inhibitors of the NF-κB alpha mutant gene (IκBαM) delivery to mensenchymal stem cells (MSCs) on rat chronic pancreatitis (CP). A total of 120 Sprague-Dawley rats were randomly divided into 6 groups of 20: Group A was injected with sterile saline solution, Group B was injected with allogenic MSCs, Group C1 was injected with allogenic IκBαM-MSCs cultured *in vitro* 4 h before CP modeling, Group C2 was injected with allogenic IκBαM-MSCs cultured *in vitro* during CP modeling, Group C3 was cultured with allogenic IκBαM-MSCs cultured *in vitro* 4 h after CP modeling, and Group D was injected with rAAV2-MSCs. Cytokine levels of ICAM-1, CTGF, IL-1, IL-6, IL-8, TNF-α, TIMP-1, TIMP-2, IL-10, FN, MMP-1, MMP-2, MMP-3, and MMP-9 were examined. The results indicated that allogenic IκBαM-MSCs could reduce pro-

inflammatory cytokine levels and increase anti-inflammatory cytokine levels in CP. The allogenic IκBαM-MSCs reduced the activation and promoted the apoptosis of pancreatic stellate cells in the rat model of CP. IκBαM-MSCs influenced the proliferation and apoptosis of pancreatic stellate cells by regulating the activation of the PPAR, MAPK, mTOR, TGF-β, NOD-like receptor, Notch, WNT, TGF-β1-SMAD-2/3, and P53 signal transduction pathways.

Key words: IκBαM; Chronic pancreatitis; Pancreatic stellate cell; Inflammatory factors; Co-cultured; Gene sequence

INTRODUCTION

In chronic pancreatitis (CP), the accumulation of extracellular matrix (ECM) causes development of pancreatic fibrosis, which results in exocrine and endocrine pancreatic insufficiency (Apte et al., 1998; Bachem et al., 1998; Phillips, 2012). Fibrosis is a potential risk factor of pancreatic cancer and may play an active role in disease progression (Haber et al., 1999). Despite significant efforts in recent years, effective therapies to inhibit or even reverse pancreatic fibrosis remain elusive.

Pancreatic stellate cells (PSCs) have recently been identified and characterized (Apte et al., 1998). Similar to hepatic stellate cells, PSCs also play key roles in pancreatic fibrosis, inflammation, and desmoplastic reactions in CP. In the normal pancreas, PSCs possessing fat droplets with vitamin A are quiescent. In the quiescent state, they are characterized by desmin-positive and α-smooth muscle actin (α-SMA)-negative staining (Apte et al., 1998). When cultured *in vitro*, the auto-activation or auto-transformation of PSCs changes their morphological and functional features (Bachem et al., 1998). PSCs start losing lipid droplets with vitamin A, show high proliferation, increased expression of α-SMA, and secretion of ECM components, such as collagen and fibronectin (FN). PSCs auto-transform to myofibroblast-like cells. PSCs are also activated during pancreatic fibrosis *in vivo* (Haber et al., 1999).

PSCs are therefore the main effector cells involved in pancreatic fibrosis (Omary et al., 2007; Phillips, 2012). Following liver damage in CP, acinar, duct, endothelial, and inflammatory cells all release a large amount of inflammatory mediators, such as platelet-derived growth factor, transforming growth factor-beta (TGF-β), interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and so on, resulting in PSC activation, aggregation, and proliferation (Apte et al., 1999; Aoki et al., 2006; Habisch et al., 2010). Finally, the cell morphology and function of PSCs are changed and self-activated to promote matrix proliferation (Apte et al., 1998, 1999; Phillips et al., 2003). A large amount of collagen is produced and deposited, eventually leading to the development of pancreatic fibrosis (Ellenrieder et al., 2004). Thus, activated PSCs are believed to play a central role in pancreatic fibrosis and are considered to be a promising target for anti-fibrotic therapies.

Nuclear factor-kappa B (NF-κB) is an important transcription factor that regulates immunity, inflammatory responses, and apoptosis. Inhibitors of NF-κB alpha (IκBα) can combine with NF-κB to keep it in an inactive state in the cytoplasm. TNF-α and IL-1 transduce signals to NF-κB inducing kinase (NIK) by binding to receptors. This in turn activates the IκB kinase (IKK) to induce the phosphorylation of Ser32 and Ser36 of IκBα. Subsequently, IκBα is ubiquitin-oriented and degraded. IκBα degradation causes dissociation of the NF-κB-IκBα

complex. Finally, free NF-κB dimers migrate to the nucleus and induce the expression of related genes (Ghosh et al., 1998). When Ser32 and Ser36 are replaced with valine residues, IκBα mutant (IκBαM) retains its binding activity to NF-κB, and phosphorylation and degradation are inhibited, which prevents NF-κB activation.

We constructed an IκBαM using valine residues instead of Ser32 and Ser36 to further block NF-κB activation in cells. In this study, we injected mensenchymal stell cells (MSCs) and IκBαM-MSCs into CP models to investigate any changes in inflammatory factors in CP. The GeneChip Rat Genome 230 2.0 array was used to investigate changes in PSC gene sequences after indirect co-culture with IκBαM-MSCs and with MSCs individually. The results of this study provide evidence for the development of therapeutic strategies for CP.

MATERIAL AND METHODS

Experimental groupings

A total of 120 Sprague-Dawley (SD) rats were randomly divided into 6 groups of 20: Group A was injected with sterile saline, Group B was injected with allogeneic MSCs cultured *in vitro*, Group C1 was injected with allogeneic IκBαM-MSCs cultured *in vitro* 4 h before CP modeling, Group C2 was injected with allogeneic IκBαM-MSCs cultured *in vitro* 4 h after CP modeling, Group C3 was injected with allogeneic IκBαM-MSCs cultured *in vitro* during CP modeling, and Group D was injected with rAAV2-MSCs.

PSCs were added to the lower layer of a Transwell system that was pre-cultured with fifth-generation MSCs, rAAV2-MSCs, and IκBαM-MSCs.

Disposal of animals during experimental procedures was performed in accordance with the guidelines and standards of treating experimental animals promulgated by The Ministry of Science and Technology of the People's Republic of China (2006).

Isolation, culture, and identification of rat bone marrow MSCs and PSC cultivation

SD rats weighing approximately 150-200 g were injected intraperitoneally with 3 mL/kg 10% chloral hydrate as anesthesia. Under sterile conditions, both ends of the rat femoral and tibial bones were cut off. Complete medium was used to flush the bone marrow cavity. After full winding and percussion mixing, large clumps were removed via lavage with a 74-μm stainless steel standard sieve. The medium was placed in 25-cm^2 culture flasks and was then incubated at 37°C in a 5% carbon dioxide (CO_2) atmosphere. The medium was changed after 48 h and then every 3 days thereafter. After 80-90% cells merged and formed a monolayer culture at the bottom of flasks, 0.25% trypsin was used for subculture. After MSCs were cultured to the fifth generation, 0.25% trypsin was used for passage. MSCs were washed twice with phosphate-buffered saline to a concentration of 2 x 10^9/L suspension, and were then filtered using a 48-μm purpose nylon mesh. Cell suspensions were poured in 2 burettes. Mouse anti-rat CD29, CD44, and CD45 monoclonal antibodies and isotype controls were added via flow cytometry in order to identify the MSC phenotype (Jia et al., 2009; Zhao and Cai, 2010).

After anaesthetizing rats with chloral hydrate, the abdominal cavity was opened under sterile conditions and the pancreas was removed. After removing the surrounding tissues of the pancreas, the pancreatic parenchyma was injected with Grey's balanced salt solution,

containing 0.02% chain enzyme protease E, 0.05% collagenase, and 0.19% DNA enzyme. The pancreas was cut into pieces and added into 50-mL enzyme digestive juice, which was then oscillated in water at 37°C for 20 min. After the enzyme digestive juice was filtered with a 74-μm steel and centrifuged at 700 *g* for 5 min, the filtrate was collected. The precipitate was centrifuged, washed twice with Hank's solution at 4°C, and then re-suspended in 10 mL Hank's solution. Subsequently, 18% Nycodenz was added to the suspension of mixed cells in a 1:2 volume. Five milliliters 18% Nycodenz, 5 mL cell suspension with Nycodenz, and 2 mL Dulbecco's modified Eagle's medium (DMEM) were added to a 15-mL centrifuge tube. After centrifugation at 1400 *g* for 5 min, a layer of white interface was found between the Nycodenz solution and the medium layer. This layer was confirmed to be PSCs. The PSCs were collected, re-suspended in high-glucose DMEM containing 20% fetal bovine serum, hydroxyethyl piperazineethanesulfonic acid, penicillin, and streptomycin, and were then incubated at 37°C in a 5% CO_2 atmosphere. The medium was changed after 24 h and then every 48 h thereafter. When 80% confluence was achieved, the cells were passaged (Apte et al., 1998; Bachem et al., 1998).

Preparation of IκBαM-MSCs

After constructing an adeno-associated virus vector carrying the IκBαM gene and infected AAV2-293 cells, the expression of the IκBαM gene was detected. rAAV2 particles were collected as described previously (Zhang et al., 2012). MSCs were transfected with IκBαM as described previously (Shelling and Smith, 1994; Pieroni et al., 1998). When MSCs reached 60 to 80% confluence, the medium was replaced with serum-free medium. Each hole was filled with 100 μL rAAV2-EGFP, which induced multiple infections reaching 10^6 cells/L. The medium was mixed gently and then incubated at 37°C in a 5% CO_2 atmosphere. After 6 to 12 h, the medium was replaced with complete medium containing serum, and the MSCs were cultured again.

IκBαM-MSCs injected into the CP model rats *in vivo*

After 60 days, the intercellular adhesion molecule-1 (ICAM-1), connective tissue growth factor (CTGF), IL-1, IL-6, IL-8, TNF-α, tissue inhibitor of metalloproteinase (TIMP)-1, TIMP-2, FN, IL-10, matrix metalloproteinase (MMP)-1, MMP-2, MMP-3, and MMP-9 levels in the rat pancreas of each group (A, B, C1, C2, C3, D) were detected.

Immunocytochemistry

Immunocytochemistry was carried out as described previously (Choi et al., 2009) using an Olympus BX51 microscope (Olympus, Tokyo, Japan). Images were digitized and then processed using the Photoshop CS5.0 software (Adobe Systems, Mountain View, CA, USA).

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Rat PSCs were isolated and cultured. After trypsinization, 100,000 cells/well were seeded on 6-well plates. After reaching subconfluence, the cells were serum-starved and stim-

ulated with no addition, 2 ng/mL TGF, 1 ng cholecystokinin, or 1 μg gastrin for 24 h. The stimulating factors were replaced after 12 h. After adding 100 μg/mL MTT (Sigma, USA), the cells were incubated for 2 h. The supernatant was removed, and 250 μL/well dimethyl sulfoxide (Sigma, USA) was added. The reaction products were transferred to microtiter plates and read in a standard microplate reader at 570 nm.

Enzyme-linked immunosorbent assay (ELISA)

Rat pancreatic tissues were rinsed with cold saline and dried with filter paper. The pancreatic tissue was ground using a stamp mill at 10,000-15,000 rpm to make a 10% homogenate. The prepared 10% homogenate was centrifuged at 3000 rpm for 10-15 min at a low temperature. Supernatant (100 μL) was extracted to determine cytokine content. After collection, the blood was allowed to stand for 1-2 h, placed in a centrifuge tube, and then centrifuged at 3000 rpm for 15 min. ELISA was performed in accordance with manufacturer instructions, provided by Shanghai Resun Biological Technology Co. Ltd.

Indirect co-culture of bone marrow MSCs and PSCs

Indirect co-culture was carried out as described previously (Zhang et al., 2012). PSCs were added to the lower layer of Transwell plates pre-cultured with fifth-generation MSCs, rAAV2-MSCs, and IκBαM-MSCs. The Transwell culture plates were placed in a heated incubator for indirect co-culture at 37°C in a 5% CO_2 atmosphere. The assembly of the Transwell non-contact co-culture system was completed following methods described (Parekkadan et al., 2011). Cell growth was observed at regular intervals.

GeneChip Rat Genome 230 2.0 array

GeneChip (Affymetrix) was used to extract the RNA of IκBαM-MSCs and PSCs. The Qiagen MidiKit was used to separate and purify mRNA. After adding reverse transcriptase, biotin-labeled cDNA probes were purified. The biotin-labeled cDNA probe was detected following the method described (Ohnishi et al., 2007) using the GeneChip Rat Genome 230 2.0 array (Affymetrix) with gene chips containing 31,099 genes (Cambon et al., 2007; Alberts et al., 2007).

Statistical analysis

Differences among groups were analyzed using one-way analysis of variance. Data are reported as means ± standard deviation and were analyzed with the SPSS 17.0 statistical software. In a two-tailed test, $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Bone marrow MSCs, PSCs, and IκBαM-MSCs

After 3 generations, the morphology of the MSCs was homogeneously fibrous (Figure 1).

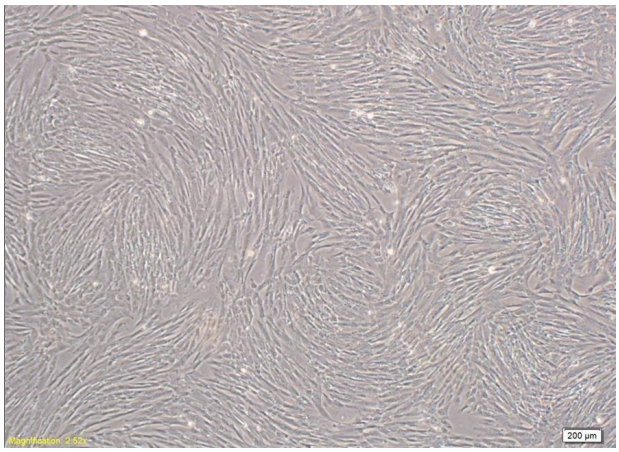


Figure 1. After 3 generations, the morphology of MSCs were homogeneously fibrous.

After 72 h, the PSCs were enlarged and had well-developed pseudopodia, showing stellate- or squid-like shapes. After stimulation by 328-nm ultraviolet light, freshly isolated cells emitted blue-green autofluorescence. α-SMA (positive staining) was observed in the cytoplasm of PSCs, which were distributed in a fibrous manner (Figure 2). We constructed a recombinant vector, pcDNA3.0-IκBαM, to transfect and package the adeno-associated virus. We used T7 BGH rev universal primers to sequence the adeno-associated virus in 2 directions (Figure 3). The transfected IκBαM-MSCs were able to emit green fluorescence under a fluorescence microscope (Figure 4).

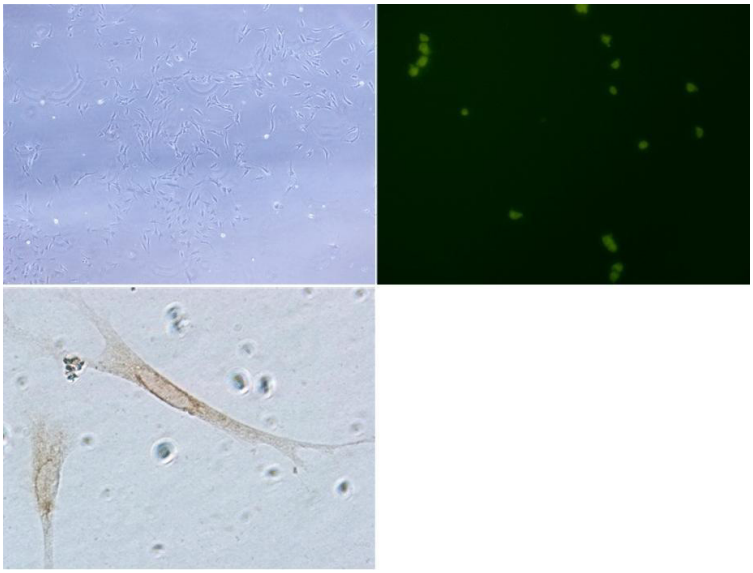
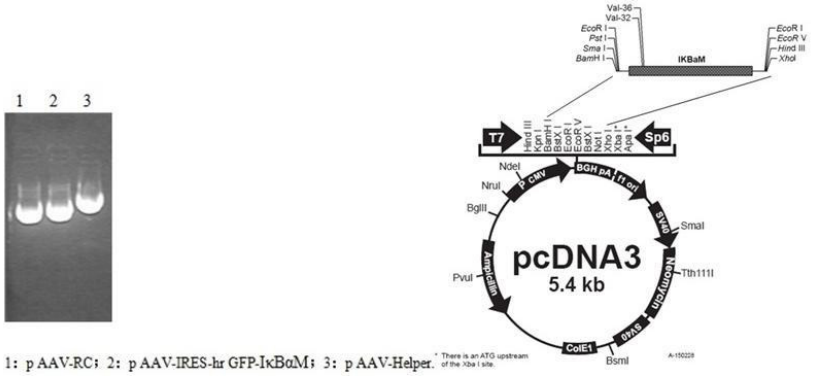


Figure 2. After 72 h, PSCs showed large and well-developed stellate-shaped or "squid-like" pseudopodia. Freshly isolated cells emitted blue-green autofluorescence after stimulation with 328 nm UV. α-SMA (positive staining) was observed in the cytoplasm of PSCs, showing a fibrous distribution.



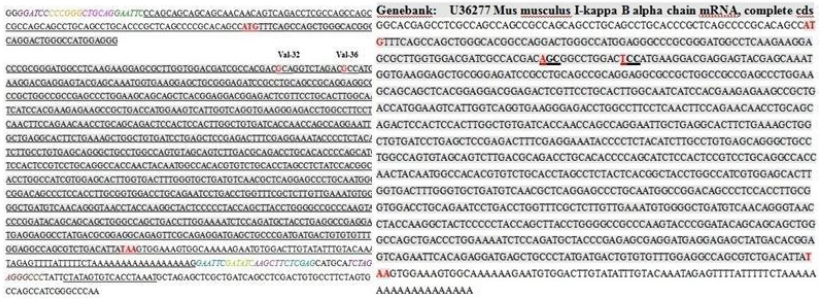


Figure 3. Results of bi-directional sequence determination of the adeno-associated virus. The T7 BGH rev universal primers were used for sequence confirmation.

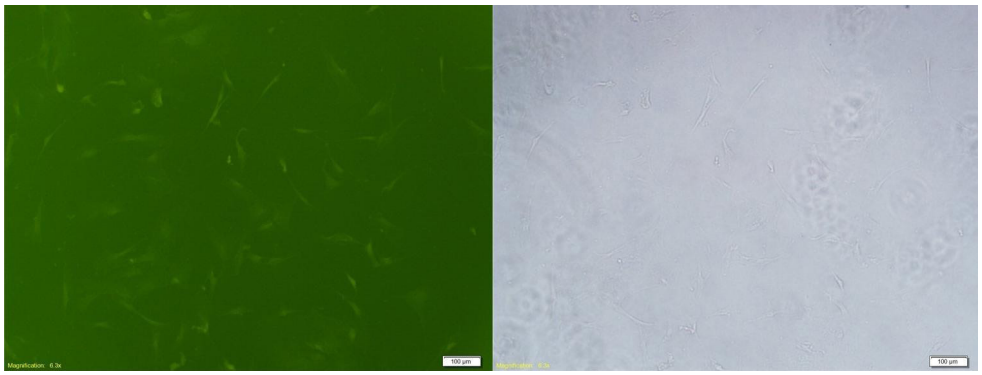


Figure 4. Transfected IκBαM-MSCs were observed to emit green fluorescence under a fluorescence microscope.

Fibrosis image of each group

The extent of fibrosis in Groups B, C, and D were less pronounced than that of Group A. The fibrosis in Groups C1, C2, and C3 were less pronounced than that of Groups B and D (Figure 5).

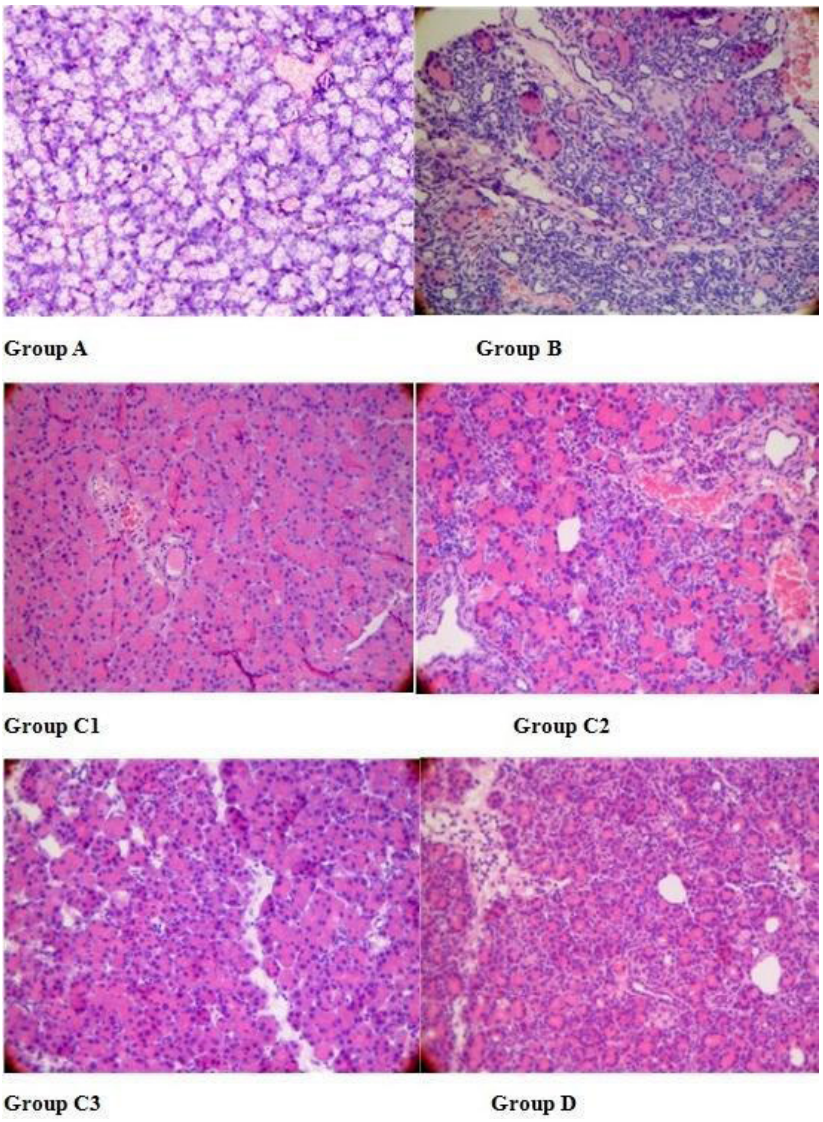


Figure 5. Fibrosis of Group B, Group C, and Group D were lower than that in Group A.

Immunohistochemical staining

The NF-κB levels in Groups B, C, and D were lower than those of Group A. The NF-κB levels in Groups C1, C2, and C3 were lower than those of Groups B and D (Figure 6). Glial fibrillary acidic protein and terminal transferase dUTP end-labeling double staining suggested PSC apoptosis in the pancreas (Figure 7).

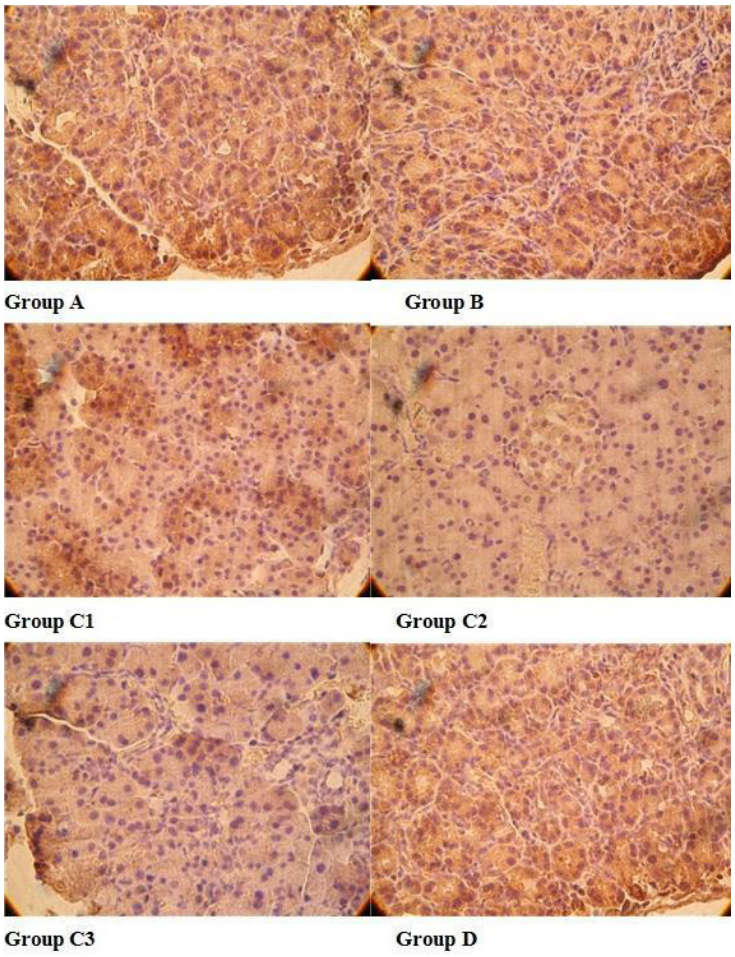


Figure 6. NF-κB in Group B, Group C, and Group D were reduced compared to that of Group A.

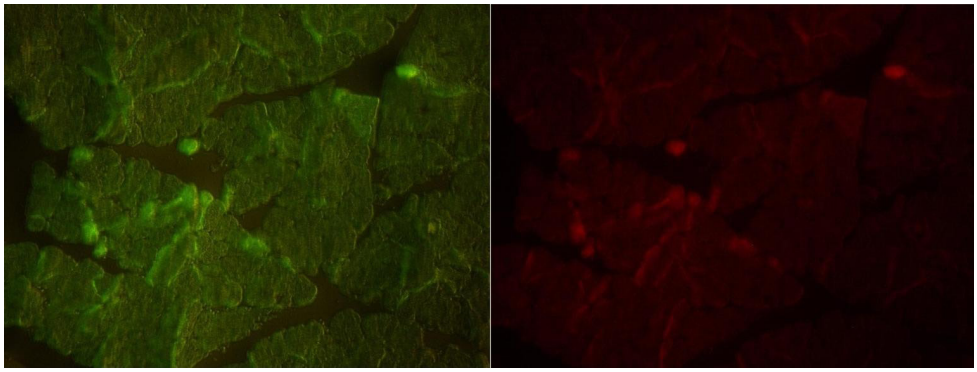


Figure 7. GFAP and TUNEL-double staining indicated apoptosis of PSC in the pancreas.

ELISA

The expression levels of IL-1, IL-6, IL-8, FN, TIMP-1, TIMP-2, TNF-α, CTGF, ICAM-1, and TGF-β_1 were lower in Group C than in Groups A, B, and D ($P < 0.05$). The expression levels of MMP-1, MMP-2, MMP-3, MMP-9, and IL-10 were higher in Group C than in Groups B and D ($P < 0.05$). No statistically significant difference was observed in expression levels between Groups B and D ($P > 0.05$). The expression levels of IL-1, IL-6, IL-8, FN, TIMP-1, TIMP-2, TNF-α, CTGF, P65, ICAM-1, and TGF-β_1 were lower in Groups B, C, and D than in Group A ($P < 0.05$). The expression levels of MMP-1, MMP-2, MMP-3, MMP-9, and IL-10 were higher in Groups B, C, and D than in Group A ($P < 0.05$).

Expression levels of IL-1, IL-6, IL-8, FN, TIMP-1, TIMP-2, TNF-α, CTGF, ICAM-1, TGF-β_1, P65, MMP-1, MMP-2, MMP-3, MMP-9, and IL-10 in rat serum are presented in Tables 1 and 2.

After the indirect co-culture of IκBαM-MSCs/PSCs and MSCs/PSCs, changes in the gene sequences of PSCs were observed in both groups. After further analysis and processing to filter biologically significant genes, some changes in signaling pathway genetic sequences were observed, including PPAR, Toll-like receptor, VEGF, hedgehog, JAK-STAT, MAPK, mTOR, TGF-β, NOD-like receptor, NOTCH, ERBB, chemokine, WNT, TGF-β_1-smad-2/3, and P53.

DISCUSSION

IκBα is an inhibitor of NF-κB that is commonly found in various cell types (Kim et al., 2012). The ankyrin repeat region of IκBα proteins combines with the Rel homology domain of NF-κB to form complexes in the cytoplasm. Such complexes prevent NF-κB from entering the nucleus to activate transcription and maintain it in a non-active state in the cytoplasm. TNF-α and IL-1 transduce signals to NIK by binding with a receptor. They then activate IKK to induce the phosphorylation of Ser32 and Ser36 in IκBα. Subsequently, IκBα is ubiquitin oriented and thus is degraded. IκBα degradation induces dissociation of the NF-κB-IκBα complex. Finally, free NF-κB dimers migrate to the nucleus to induce the expression of related genes.

The NF-κB signaling pathway plays an important role in CP development. We constructed an IκBα mutant to block the activation of NF-κB in CP to provide further evidence that IκBαM could be useful for treating CP. We replaced Ser32 and Ser36 with valine residues. IκBαM retained its binding activity to IκB without inducing phosphorylation and degradation. Subsequently, NF-κB could not be activated. Several studies have reported that MSCs are useful in repairing damaged organization and in promoting rebirth. This study was therefore designed to specifically examine the role of IκBαM-MSCs in the treatment of CP.

Several studies have indicated that blocking the NF-κB signaling pathway and injecting MSCs can regulate inflammatory factors and apoptosis of PSCs in acute pancreatitis. The results of the present study suggest that these events can also occur in CP. In this study, we found that inflammation in the pancreas of the IκBαM transfection group was significantly reduced compared to that of the pancreatitis group. This result suggests that IκBαM inhibited the inflammatory response in the early period of CP and played a protective role in the occurrence and development of CP.

Table 1. Expression levels of IL-1, IL-6, IL-8, FN, TIMP-1, TIMP-2, TNF-α, CTGF, ICAM-1, TGF-β_1, and P65 in rat serum are described.

Group	IL-1 (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	TNF-α (ng/mL)	NF-κB (p65) (pg/mL)	FN (μg/mL)	CTGF (ng/mL)	ICAM-1 (ng/mL)	TGF-β1 (pg/mL)	TIMP-1 (ng/mL)	TIMP-2 (ng/mL)
A	911.037 ± 31.674	13.402 ± 1.152	648.359 ± 39.467	332.50 ± 121.15	1123.267 ± 162.326	309.311 ± 62.408	883.382 ± 106.552	374.264 ± 55.237	782.926 ± 139.149	54.078 ± 2.81	195.377 ± 85.659
B	730.563 ± 65.348	9.836 ± 1.133	371.542 ± 82. 325	278.50 ± 112.45	628.530 ± 191.327	156.248 ± 55.331	653.049 ± 106.4228	192.861 ± 67.042	463.229 ± 59.461	32.353 ± 3.22	94.263 ± 74.362
C											
C1	623.315 ± 35.437	6.067 ± 1.090	317.523 ± 69.340	223.45 ± 91.38	442.035 ± 102.433	126.463 ± 19.569	476.055 ± 87.354	157.259 ± 66.246	291.334 ± 68.984	21.096 ± 3.44	63.145 ± 6.442
C2	632.347 ± 36.267	5.977 ± 1.095	322.322 ± 66.332	215.659 ± 91.25	426.305 ± 94.320	111.093 ± 22.316	434.188 ± 69.548	146.039 ± 55.241	301.628 ± 54.367	22.127 ± 4.58	58.824 ± 7.903
C3	637.427 ± 36.531	6.087 ± 1.093	312.487 ± 65.089	237.45 ± 88.36	405.209 ± 103.697	92.381 ± 26.865	455.855 ± 82.984	123.409 ± 38.797	287.872 ± 43.294	21.124 ± 5.03	51.062 ± 8.820
D	735.337 ± 70.433	9.468 ± 1.277	377.483 ± 86.325	288.14 ± 121.74	628.530 ± 191.327	149.348 ± 88.484	682.466 ± 177.818	188.133 ± 62.566	472.531 ± 89.237	34.197 ± 4.06	96.082 ± 7.034

Table 2. Expression levels of MMP-1, MMP-2, MMP-3, MMP-9, and IL-10 in rat serum are described.

Group	IL-10 (pg/mL)	MMP-1 (ng/mL)	MMP-2 (ng/mL)	MMP-3 (ng/mL)	MMP-9 (ng/mL)
A	314.325 ± 31.067	309.411 ± 18.479	286.346 ± 22.764	412.236 ± 55.294	264.339 ± 36.195
B	590.094 ± 55.237	388.934 ± 50.363	367.422 ± 17.992	645.331 ± 150.424	326.526 ± 70.465
C					
C1	683.289 ± 90.276	732.227 ± 23.561	420.351 ± 30.438	1102.394 ± 70.665	904.589 ± 27.359
C2	674.035 ± 88.274	741.465 ± 14.326	395.834 ± 19.665	1034.287 ± 76.009	887.638 ± 39.442
C3	680.799 ± 91.624	759.306 ± 12.744	411.371 ± 15.279	1206.267 ± 102.381	979.355 ± 55.367
D	586.471 ± 60.372	391.894 ± 38.452	394.117 ± 25.864	676.953 ± 172.135	314.885 ± 83.204

Pancreatic fibrosis is mainly caused by the imbalance between the synthesis and deposition of the ECM in the pancreatic tissue. Type I collagen, type III collagen, and FN are all integral components of the ECM in the pancreatic tissue (Akita et al., 2012; Yan et al., 2012). Reduction of these 3 factors can prevent deposition of the ECM in the pancreatic tissue and mitigate pancreatic fibrosis. The results showed that IκBαM-MSCs reduced the amounts of type I collagen, type III collagen, and FN in rats. The pathological specimens also suggested a significant difference in pancreatic fibrosis among groups. The pancreatic fibrosis in Groups C1, C2, and C3 was less pronounced than that in Groups A, B, and D.

MMPs, such as MMP-1, MMP-2, MMP-3, and MMP-9, can promote degradation of the ECM in the pancreas while inhibiting expressions of TIMP-1 and TIMP-2 (Prakash et al., 2011; Ko et al., 2012). The results of the present study suggested that IκBαM-MSCs can increase expression levels of MMP-1, MMP-2, MMP-3, and MMP-9 in the pancreatic fibrosis tissue and reduce those of TIMP-1 and TIMP-2. Pancreatic fibrosis was also improved in some pancreatic tissues of Groups C1, C2, and C3.

In this study, the expression levels of IL-10 and TGF-β_1 increased significantly in Groups C1, C2, and C3 relative to Groups B and D. Therefore, blocking the NF-κB signaling pathway can increase expression levels of IL-10 and TGF-β_1. Several studies have suggested that as a secreted protein, CCN, the CTGF can stimulate cell mitosis, chemotaxis, proliferation, and ECM formation (Grotendorst et al., 2000). Abreu et al. (2002) found a synergistic relationship between CTGF and TGF-β_1. IL-10 is an effective antifibrotic cytokine. Endogenous IL-10 may limit pancreatic gland atrophy and fibrosis as well as reduce the expression level of TGF-β_1 in pancreatic acinar cells and interstitial cells (Bendicho et al., 2005; Apte et al., 2007).

Several studies (Rakonczay Jr. et al., 2008) have shown that over-activation of NF-κB in the early course of CP can increase expression levels of many pro-inflammatory cytokines, inflammatory mediators, adhesion molecules, and acute phase proteins, all of which can promote inflammation, activate PSCs, and accelerate pancreatic fibrosis in CP. Therefore, inhibition of inappropriate activation of NF-κB in the early stage of CP could reduce the overproduction of inflammatory mediators and cytokines. Thus, the initial stage of CP can be inhibited to a certain extent in order to delay its further development.

TGF-β_1 is a multifunctional cytokine that combines with its receptors, RI and RII, at the cell surface to form complexes and regulate cell growth and differentiation (Wang et al., 2012). TGF-β_1 complexes play important roles in tissue fibrosis and repair by stimulating cell matrix synthesis and inhibiting degradation. TGF-β_1 can activate PSC to induce fibroblast differentiation and secretion of various components of the ECM, such as type I collagen and FN. Several studies have revealed that PSCs can induce TGF-β_1 synthesis. Therefore, PSCs may be the major source of TGF-β_1 in the pancreatic tissue. We found that IκBαM-MSCs can

reduce the expression level of TGF-β_1. Through gene sequence detection, we found that the TGF-β_1, Smad2/3, CycA, and ORC genes of PSC sequences were differentially expressed after co-culture with IκBαM-MSCs. Ohnishi et al. (2004) found that TGF-β_1 promotes the activation of PSCs through the Smad2-dependent pathway and that PSCs infected by the adenovirus with the Smad2-dominant-negative gene inhibit PSC activation. IκBαM-MSCs can reduce the secretion of TGF-β_1, thereby inhibiting the transduction of the TGF-β_1-smad-2/3 signaling pathway. This process can affect DNA biosynthesis and inhibit the proliferation of PSCs, which benefit the treatment of CP.

In our previous study (Liu and Qin, 2011), we confirmed that PSCs play key roles in CP; however, the influencing factors are complex and difficult to clearly analyze *in vivo*. To further investigate the relationship between IκBαM-MSCs and CP, the relationship between IκBαM-MSCs and PSCs should be investigated. A Transwell culture plate is an indirect culture system that can be used to observe the effect of IκBαM-MSCs on PSCs. Further studies using ELISA and the GeneChip Rat Genome 230 2.0 array are currently underway to determine the mechanism linking IκBαM-MSCs and PSCs. The *in vitro* test of the present study revealed the possible signaling pathway regulating the inflammatory factors and apoptosis of PSCs.

Extracellular signal-regulated protein kinase, p38, and c-Jun N-terminal protein kinase are 3 important members of the MAPK superfamily. MAPK plays an important role in PSC activation (McCarroll et al., 2003). The MAPK signaling pathway was downregulated in IκBαM-MSCs/PSCs, especially p38.

The encoded product of the p53 gene is composed of 393 amino acids divided into WTP53 and MTP53. WTP53 promotes apoptosis, reduces the expression level of the endogenous bcl-2 protein, and inhibits its function by downregulating the bcl-2 gene. WTP53 increases the target gene *bax* to improve the expression of the intracellular bax protein, which induces an imbalance in the bcl-2/bax protein and promotes apoptosis. Pei et al. (2008) found that NF-κB may inhibit cell apoptosis by upregulating the expression of the bcl-2 gene. In this study, Reprimo, Gadd45, and Cyclin B gene fragments were expressed through the p53 signaling pathway. Therefore, IκBαM may arrest the cell cycle through the Reprimo/Gadd45-Cyclin B signaling pathway. Moreover, PERP, CytC, and IGF gene fragments showed genetic variations. Combined with previous studies, we can speculate that IκBαM induces PSC apoptosis through the P53-IGF and P53-PERP-CytC-CASP9-CASP3 signaling pathways.

Our previous study (Zhang et al., 2009) showed that the self-activation of PSCs can be limited and apoptosis of PSCs can be induced by inhibiting the activation of NF-κB in PSCs. Therefore, IκBαM inhibits the factors influencing CP and reduces pancreatic fibrosis. Combined with results of the present study, IκBαM-MSCs were proven to play significant roles in controlling inflammatory factors and pancreatic fibrosis of CP.

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