Human embryonic stem cell-derived mesenchymal stem cells ameliorate severe acute pancreatitis in a rat model

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Directed by Professor **Seungmin Bang**

Doctoral Dissertation
submitted to the Department of Medicine
at the Graduate School of Yonsei University,
in partial fulfillment of the requirements for the
degree of Doctor of Philosophy

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June 2020

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June 2020

ACKNOWLEDGEMENTS

I am grateful to a number of people who supported me through this thesis. First of all, I would like to express my deep gratitude to Professor Seungmin Bang for his continuous support, patience, and immense knowledge which he shared throughout my research career, and my life as physician. His guidance encouraged and inspired me in throughout this thesis. I also would like to thank my thesis committee: Professors Chang Moo Kang, Hyun Ok Kim, Jeong Youp Park, and Jae Hee Cho for their insightful comments and for encouraging me to widen my research from various perspectives. I'm also grateful to researcher Yoo Keung Tae, who provided full support for the experiment, and Professor Ji Hae Nahm, who helped to read the pathology. Last but not least, I would like to thank my lovely family: my father Ki-Young, my mother Hee-Youn, my sister Min-Seo, my wife Yuri, and my son Yoon-Woo, for giving me boundless love, consistent trust, and spiritual support throughout writing this thesis, and in my life in general.

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ABSTRACT

Human embryonic stem cell-derived mesenchymal stem cells ameliorate severe acute pancreatitis in a rat model

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Background: Severe acute pancreatitis (SAP) is a fatal disease with high mortality. Mesenchymal stem cells (MSCs) are possible therapeutics for SAP. However, human embryonic stem cell-derived MSCs (hES-MSCs) have not yet been studied for the treatment of SAP.

Methods: SAP was induced in Sprague-Dawley rats by intraductal injection of 3% sodium taurocholate solution. After SAP induction, survival rates and pathologic change of pancreas were compared between hES-MSCs infused and normal saline (NS) infused rats. Serum inflammatory cytokines levels were measured via enzyme-linked immunosorbent assay. Immunofluorescence stain with anti-Human Nuclear Antigen-antibody and luciferase transfected

hES-MSCs (hES-MSC-Luc) were used to trace the biodistribution of MSCs.

Results: Compared to NS infused SAP rats, hES-MSC infused SAP rats had significantly higher survival rate (18/26 vs 13/13 at 24 hours, $p = 0.025$, and 10/20 vs 8/8 at 48 hours, $p = 0.013$) and reduced serum amylase and lipase levels (6930 ± 1440 vs 2708 ± 735 at 48 hours, $p=0.022$ and 1126 ± 107 vs 786 ± 87 at 48 hours, $p = 0.049$). Also, pathology scores of the pancreas showed that hES-MSC infusion improved pancreatitis compared to NS infusion (8.2 ± 1.2 vs 7.0 ± 0.9 at 24 hours, $p = 0.019$, and 8.0 ± 1.2 vs 5.8 ± 1.4 at 48 hours, $p < 0.001$). Immunofluorescence revealed that hES-MSCs were present in the lung of SAP rats, but not in the pancreas. *In vivo* biodistribution tracing also confirmed that hES-MSC-Luc was trapped in the lung without uptake observed in the pancreas. IL-1β, IL-6, INF-γ, and TNF-α decreased significantly following hES-MSC infusion in SAP rats, compared to NS.

Conclusions: hES-MSC therapy was safe and effectivly reduce pancreatic damage and mortality in a SAP rat model. hES-MSCs function seemed to work as an immunomodulator via inhibiting inflammatory cytokines. These findings suggested that the hES-MSC might be a potential therapeutic agent for SAP in human.

Key words: severe acute pancreatitis, human embryonic stem cell-derived mesenchymal stem cell, rat model

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I. INTRODUCTION

Acute pancreatitis is a disease with an annual prevalence of about 40 out of 100,000 people globally. Approximately 20% of patients with acute pancreatitis progress to severe disease, 10% to 30% of whom die.[1] Despite the rapid development of intensive therapy for critically ill patients, in the past decades, severe acute pancreatitis (SAP) has not shown a significant reduction in mortality.[2] It is widely accepted that autodigestion of the pancreas occurs due to the premature activation of pro-enzymes in pancreatic acinar cells.[3] In addition, inflammatory substances such as Interleukin (IL)-1, IL-6, IL-8, C-reactive

protein, tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), nitric oxide, and endothelin are related to the occurrence and progression of pancreatitis. Although some understandings have been made on the mechanism of acute pancreatitis, progress in the development of effective treatment for SAP is slow.

Mesenchymal stem cells (MSCs) are capable of differentiating into various cells and restoring damaged cells in lung injury, renal failure, and myocardial infarction.[4-7] They also have an immunomodulatory function that inhibits inflammatory damage in diseases such as systemic sclerosis, Crohn's disease, and diabetes.[8-12] Considering the pathogenesis of acute pancreatitis, it is entirely plausible that that MSCs may contribute to reducing the severity of the disease and lowering its mortality rate by restoring tissue damage in the pancreas and inhibiting the expression of inflammatory substances. In animal studies, MSCs transplanted in acute pancreatitis-induced rats induced recovery of damaged pancreatic tissue and reduced expression of inflammatory substances.[13,14]

However, standard preparations of human adult MSCs generally derived from umbilical cord, adipose tissue, or bone marrow, have several problems. First, the human adult MSCs have limited differentiation potential and self-renewing capacity. The quality of MSCs might be compromised by aging and own underlying diseases.[15] Second, harvesting MSCs from adult tissue requires suitable donors and invasive procedures. The number of MSCs that can be

harvested from a single donor is limited. In addition, extensive culture time increases the potential risk for chromosomal aberrations.[16,17] Furthermore, cultured-derived human adult MSCs are heterogeneous due to donor diversity.[18]

The human embryonic stem cell-derived mesenchymal stem cell (hES-MSC) is characterized by its excellent proliferative capacity, suitability for large-scale cultivation with various differentiation ability, and immunomodulatory cytokine secretion function.[19-21] hES-MSC preparation does not require invasive procedures and can be harvested in a large amount from a single donor, reducing donor diversity. All these characteristics make hES-MSCs a clinically attractive treatment option for SAP.

A variety of MSCs have been applied to animal models for the treatment of pancreatitis, but no studies have been conducted on the therapeutic effects of hES-MSC. The purpose of this study was to evaluate the safety and therapeutic efficacy of hES-MSC therapy for SAP in a rat model.

II. MATERIALS AND METHODS

1. Human embryonic stem cell-derived mesenchymal stem cell

The hES-MSCs used in the current study were developed by Daewoong Pharmaceuticals. Co., Ltd (Seoul, Republic of Korea). hES-MSC lines were derived from human embryonic stem cells (ESC) by EB-based differentiation methods. Derivation methods are described elsewhere.[22] (Korean Patent

Application No. 10-2019-0162393)

hES-MSCs used for experiments in the current study were received via a Research Agreement Contract. hES-MSCs were delivered in frozen vials (5×10^6 cells/mL/vial) in CS10 media (BioLife Solutions, Owego, NY, US). After thawing and washing, hES-MSC vials were transferred to normal saline (NS) excipient. After cell counting, cells were administered (5×10^6 cells/kg) to rat models.

2. Characterization of hES-MSC

hES-MSC characterization was performed by using the modified minimal criteria of MSC which was reported by International Society for Cellular Therapy (ISCT).[23] Cell surface markers were analyzed by flow cytometry (BD FACSVerseTM, BD Bioscience, San Jose, CA, US) using human monoclonal antibodies. Mesenchymal stem cell surface markers (positive markers) were analyzed using anti-CD29-PE-conjugate, anti-CD44-PE-conjugate, anti-CD73-PE-conjugate, and anti-CD105-PE-conjugate. Hematopoietic lineage markers (negative markers) were analyzed using anti-CD34-FITC and anti-CD45-FITC conjugate. MHC class type II markers (negative markers) were analyzed using anti-HLA-DR-FITC conjugated antibodies. In addition, pluripotent stem cell markers (negative markers) were analyzed using anti-SSEA3-PE-conjugate, anti-Tra-1-60-FITC conjugate, and anti-Tra-1-81-FITC conjugate. All antibodies were purchased from BD

Bioscience (San Jose, CA, US), and their signals were analyzed by comparing fluorescence intensities and IgG type matched-isotype controls.

The differentiation potential of ES-MSCs was investigated by tri-lineage differentiation, adipocyte, osteocyte, and chondrocyte. Adipocyte and osteocyte and chondrocyte differentiation processes were performed by commercialized differentiation conditioned media (Gibco, Rockville, MD, US) according to the manufacturer's recommendations. Differentiated cells were confirmed by Oil-red-O stain (adipocyte), Alizarin Red S stain (osteocyte), and Alcian Blue stain (chondrocyte).

3. SAP induction in the rat model

Male Sprague-Dawley rats (OrientBio, Seongnam, Republic of Korea) weighing 250-300 g were used for the experiments. Animal care and all experimental procedures were conducted in accordance with the Guide for Yonsei University Health System Institutional Animal Care and Use Committee.

For comparison study, rats were divided into a control group; which is normal rats did not receive SAP induction procedure, and an SAP group; which received SAP induction procedure. The control group and SAP group were randomly received hES-MSCs or NS via tail vein. Nomenclatures of subgroups were as following: Control group which received NS or hES-MSC; Control-NS or Control-ES, SAP group which received NS or hES-MSC; SAP-NS or

SAP-ES. The total number of rats in each group was determined to be the number of at least 5 samples may obtain at 24hr and 48hr after SAP induction depending on the survival rate of each group. SAP was induced in rats following a previous reported surgical method.[24] Rats were anesthetize with isoflurane (2-4 vol%), and the abdominal midline was opened. The proximal and distal portions of the biliary duct were temporarily clipped and 1 ml/kg of 3% taurocholate (TC) was slowly infused for 1 min between the clipped biliary tract to induce SAP. After release of the temporary biliary clamp, the peritoneum and abdomen were closed and sutured. Antibiotics (gentamycin, 3 mg/kg) were administered before the procedure as an intramuscular injection at thigh muscle and analgesics (ketorolac, 1 mg/kg) were administered after the procedure as same route.

4. hES-MSC injection to rat model

After induction of SAP, the rats were divided randomly into two groups: SAP with NS (SAP-NS group), and SAP with hES-MSC (SAP-ES group). Our preliminary study indicated that the mortality rate of the SAP rat model induced by the surgical procedure used in this study was about 30%. The mortality rate decreased to about 10% with human umbilical cord derived MSC therapy (data not shown). Therefore, according to the predicted mortality, we assigned 26 rats to the SAP-NS sub-group and 13 rats to the SAP-ES sub-group. NS 1 mL or hES-MSCs (5×10^6 cells/kg) suspended in

NS 1mL, respectively, were injected via the tail vein. Rats were planned to sacrifice by decapitation at 24 h, 48 h or 1 week after SAP induction. Blood was collected at 24 h and 48 h. In cases of live rats, approximately 0.5 mL of blood was collected from alive rats via the retro-orbital plexus. In cases of sacrifice, the thoracic cavity was opened and about 2-3 mL of blood was collected directly from the heart. After sacrifice, the pancreas, lungs, and liver were harvested to make frozen and formalin-fixed paraffin-embedded (FFPE) blocks for pathologic evaluation. All processes were performed under isoflurane (2-4 vol%) anesthesia. Collected blood was centrifuged (500 g, 25 minutes, 4℃) to extract serum for cytokine detection.

5. Pathologic evaluation after hES-MSC injection to SAP rat model

Pancreas sections were subject to hematoxylin and eosin (H&E) stain and scored for evaluation of pathologic damage. 20 randomly chosen microscopic fields with 200 x magnification were scored as previous described.[13,14,25] Pancreatic sections were graded for the extent and severity of interstitial edema, parenchymal necrosis and Infiltration of inflammatory cells according to the score provided in Table 1. The average scores of each group were compared to determine the therapeutic effect of hES-MSC treatment. Pathologic scoring was independently performed by the main researcher (JH Jo) and a pathologist (JH Nam) who were blinded to pancreatic enzyme levels and the type of treatment.

Table 1. Pancreas Histologic Scoring[13,14,25]

1. Interstitial edema score

Score	Features
0	Absent
1	Expanded interlobular septa
2	Expanded intralobular septa
3	Separated individual acini

2. Parenchymal necrosis

Score	Features
0	Absent
1	periductal necrosis (<5%),
2	focal necrosis (5-20%)
3	Diffuse parenchymal necrosis (20-50%)

3. Infiltration of inflammatory cells

Score	Features
0	Absent
1	present in ducts (around ductal margins)
2	present in the parenchyma (in <50% of lobules)
3	present in the parenchyma (in >50%of lobules)

6. Tracking of hES-MSC *in vivo* after luciferase gene transduced hES-MSC (hES-MSC-Luc)

hES-MSC were labeled with commercially available firefly luciferase lentiviral vector (Redifec^{TM} Red-Fluc-Puro, Perkin Elmer, Boston, MA, US) and Lentiboost^{TM} (SIRION Biotech, Martinsried, Germany). All assays were performed according to the manufacturers' instructions.

Linearity was used *in vivo* for analysis of luciferase gene introduced hES-MSCs (hES-MSC-Luc, PN=15, Daewoong Pharmaceuticals. Co., Ltd., Republic of Korea) using an IVIS spectrum CT system (Perkin Elmer, Boston, MA, US). To confirm the linearity of hES-MSCs introduced with firefly luciferase gene, hES-MSC-Luc cells were subcutaneously administered to six points of dorsal skin in normal mice (starting at 1×10^5 cells and serially diluted to 6.25×10^3 cells). Luminescence images were obtained using the IVIS spectrum CT system after administration of luciferin (Perkin Elmer, Boston, MA, US).

In vivo tracking of hES-MSC was performed using transfected hES-MSC-Lucs (PN=15, 5×10^6 cell/kg) which were infused via the tail vein of SAP rats. After intraperitoneal injection of luciferin (PerkinElmer, MA, US), luminescence images were analyzed. The *in vivo* distribution of MSCs over time was tracked using the Caliper IVIS Lumina II *in vivo* imaging system (Caliper Life Sciences, Hopkinton, MA, US).

7. Immunofluorescence staining

Tissue slides were deparaffinized in xylene and rehydrated in graded alcohol. Endogenous peroxidase activity was blocked with 0.3% (v/v) hydrogen peroxide in methanol. Antigen retrieval was performed by microwaving the slides in sodium citrate buffer (0.01 M, pH 6.0) for 5 min. To block nonspecific staining, sections were incubated with 10% (v/v) normal donkey serum for 1 h and incubated with mouse monoclonal anti-Human Nuclear Antigen-antibody (Dilution 1:100, Abcam, Cambridge, UK) and rabbit polyclonal anti-PDX1 antibody (Dilution 1:200, Abcam, Cambridge, UK) overnight at 4 ℃. Slides were visualized using Alexa488 goat anti-mouse IgG2a (Human Nuclear Antigen, 1:500, Jackson ImmunoResearch Inc.) and Alexa555-Donkey anti-rabbit IgM (PDX1, 1:500, Jackson ImmunoResearch Inc.) in antibody diluent, and incubated for 30 min at room temperature. Between each step, washing was performed thrice, for 5 min each on a rocking platform in PBS. The slides were coverslipped using mounting medium for fluorescence with DAPI (Vecta shield H-1200; Vector Laboratories, Inc. Burlingame, CA, US). Stained slides were analyzed using an Olympus BX53 microscope and images were captured using an Olympus DP71 camera (Olympus America Inc., Center Valley, PA, US).

8. Serum amylase, lipase, and inflammatory cytokines measurement

Serum amylase levels were measured with a commercial FUJI DRI-CHEM

4000i analyzer (Fujifilm, Tokyo, Japan). Serum lipase levels were measured with a commercial QuantiChrom™ Lipase Assay Kit (BioAssay Systems, CA, US). All assays were performed according to the manufacturers' instructions.

Using an ELISA purchased from R&D Systems (MN, US), Serum IL-6 (Cat. No. SR6000B), IL-1β (Cat. No. SRLB00), TNF-α (Cat. No. SRTA00), TFG- β1 (Cat. No. SMB100B), and IFN-γ (Cat. No. SRIF00) levels were measured. All assays were performed according to the manufacturers' instructions.

9. Statistical analysis

Data were analyzed using $\chi 2$ and Fisher's exact test for categorical data and Student's t-test and Mann-Whitney U test for continuous variables. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, US). A value of $p < 0.05$ was considered statistically significant.

III. RESULTS

1. Characteristics of hES-MSC

Under the microscope, hES-MSCs had canonical mesenchymal stem cell morphology with a fibroblast-like spindle shape (Figure 1A). FACS analysis showed that hES-MSCs was CD29 (+), CD44 (+), CD73 (+), and CD105(+) as mesenchymal stem cell markers. hES-MSCs was CD34 (-) and CD45 (-), both of which were hematopoietic stem cell markers;

HLA-DR (-), MHC Class II markers; and Tra-1-60 (-), Tra-1-81 (-), and SSEA-3 (-), pluripotent stem cell markers (Figure 1B). Tri-lineage differentiation potential was confirmed by chondrogenesis, osteogenesis, and adipogenesis Cell type-specific staining showed that hES-MSCs successfully differentiated into chondrocytes, osteoblasts, and adipocytes *in vitro*. (Figure 1C). These results indicated that the hES-MSC had typical properties of MSC and differentiation potential for multiple mesenchymal derivatives.

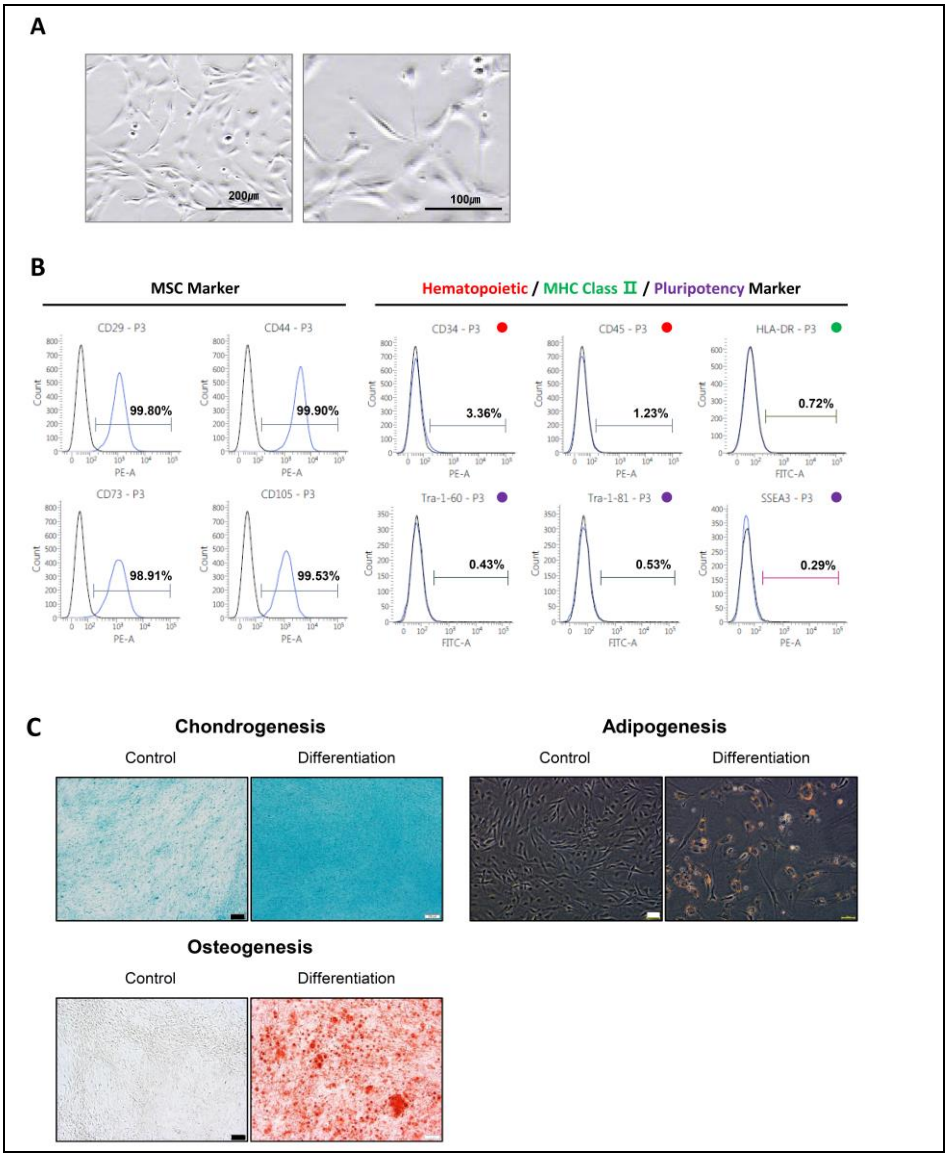

Figure 1. Characteristics of human embryonic stem cell-derived mesenchymal stem cell (hES-MSC). (A) Representative morphologies of human embryonic stem cell-derived mesenchymal stem cell (hES-MSC) (Passage number = 10). hES-MSC showed canonical mesenchymal stem cell morphology like fibroblasts (spindle-shaped). **(B)** Cell surface marker analysis of hES-MSCs (PN=10). CD29, CD44, CD73, and CD105 were analyzed for mesenchymal stem cell markers. CD34 and CD45 were analyzed for hematopoietic stem cell markers. HLA-DR was analyzed for MHC Class II markers, Tra-1-60, Tra-1-81. SSEA-3 was analyzed for pluripotent stem cell makers. **(C)** Tri-lineage differentiation potential was confirmed by chondrogenesis, osteogenesis, and adipogenesis.

2. Establishing an SAP rat model

To establish control and SAP models for comparison, rats were divided into the control group and an SAP group. First, the control group (without SAP induction; n=10) received NS (Control-NS, n=5) or hES-MSC (Control-ES, n=5) injection via the tail vein (Figure 2A). The control group was sacrificed 24 h after NS or hES-MSC injection and organs and blood samples were obtained. For the SAP group, in 39 out of 45 rats (86.7%) SAP was successfully induced. (Figure 2A). Thirty-nine SAP rats received either NS (SAP-NS, n=26) or hES-MSC (SAP-ES, n=13) infused via the tail vein.

The SAP group was observed until 48 h after SAP induction to measure survival rate (Figure 2B). After SAP induction , five rats from the SAP-NS group and six rats from the SAP-ES group were sacrificed at 24 h; and six rats from the SAP-NS group and five rats from the SAP-ES group were sacrificed at 48 h to obtain organ and blood samples. Rats that survived after 48 h were sacrificed 1 week or 2 weeks after procedures.

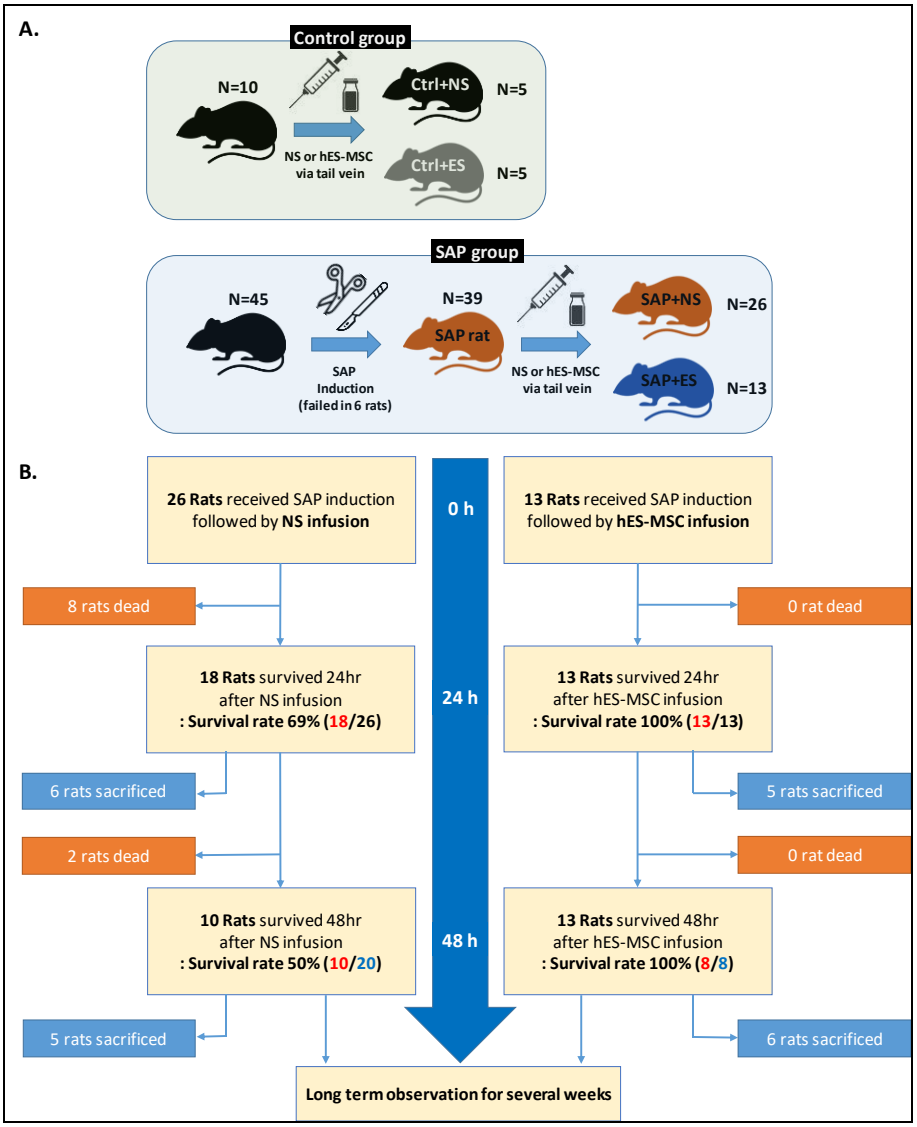

Figure 2. Experimental scheme. (A) Schematic of the experiment process according to the control group and severe acute pancreatitis (SAP) group. Each group received normal saline (NS) or human embryonic stem cell-derived mesenchymal stem cell (hES-MSC) via the tail vein. **(B)** Follow-up schedule of SAP group after NS or hES-MSC infusion. Expired rats represents mortality of SAP. A number of survived rats at 24 h and 48 h after SAP induction were sacrificed to obtain organs and blood samples.

3. Rat survival and pancreatic enzyme levels according to experimental groups

We evaluated the survival of the SAP group at 24 h and 48 h after SAP induction and NS (SAP-NS) or hES-MSC infusion (SAP-ES). Sacrificed rats at 24 h were excluded from survival analysis for 48 h. Until 48 h after SAP induction, there was no mortality in the SAP-ES group. However, in the SAP-NS group, eight rats died within 24 h and two more rats by 48 h. Survival rates were significantly higher in the SAP-ES group compared to the SAP-NS group (100% vs. 69.2%, $p = 0.025$ at 24 h; 100% vs. 50%, $p = 0.013$ at 48 h, Table 2 and Figure 3A).

Baseline amylase and lipase levels were not different between SAP-NS and SAP-ES groups (1877 vs 2089 U/L, $p=0.137$; 1346 vs 1606 U/L, $p = 0.336$). Amylase and lipase were elevated 24 h after SAP induction in both groups. Amylase elevated from 1877 to 8755 U/L in SAP NS group ($p < 0.0001$) and from 2089 to 7104 U/L in SAP-ES group ($p = 0.0001$). Lipase elevated from 1346 to 2553 U/L in SAP NS group ($p = 0.036$) and from 1606 to 2211 U/L in SAP-ES group ($p = 0.081$). Amylase and lipase became lower in the SAP-ES group compared to the SAP-NS group at 48 h ($p = 0.022$ and $p = 0.049$, respectively, Table 2, Figure 3B and C). These results suggested hES-MSC treatment could reduce mortality rates and serum pancreatic enzyme levels in the SAP induced rat model.

Table 2. Comparison of survival and serum amylase/lipase level of SAP rats according to treatment type

		SAP-NS	*SAP-ES*	*P value*
Survival, % (N)	**24 h**	69.2% (18/26)	100% (13/13)	0.025*
	48 h	50% (10/20)	100% (8/8)	0.013*
Amylase, mean U/L (SEM)	**0 h**	1877 (114)	2089 (80)	0.137
	24 h	8755 (506)	7104(1189)	0.186
	48 h	6930 (1440)	2708 (735)	0.022*
Lipase, mean U/L (SEM)	**0 h**	1346 (220)	1606 (127)	0.336
	24 h	2553 (428)	2211 (276)	0.521
	48 h	1126 (107)	786 (87)	0.049*

Abbreviation : SEM; standard error of the mean, SAP-NS; Severe acute pancreatitis-normal saline injected group, SAP-ES; Severe acute pancreatitis-human embryonic stem cell derived mesenchymal stem cell injected group.

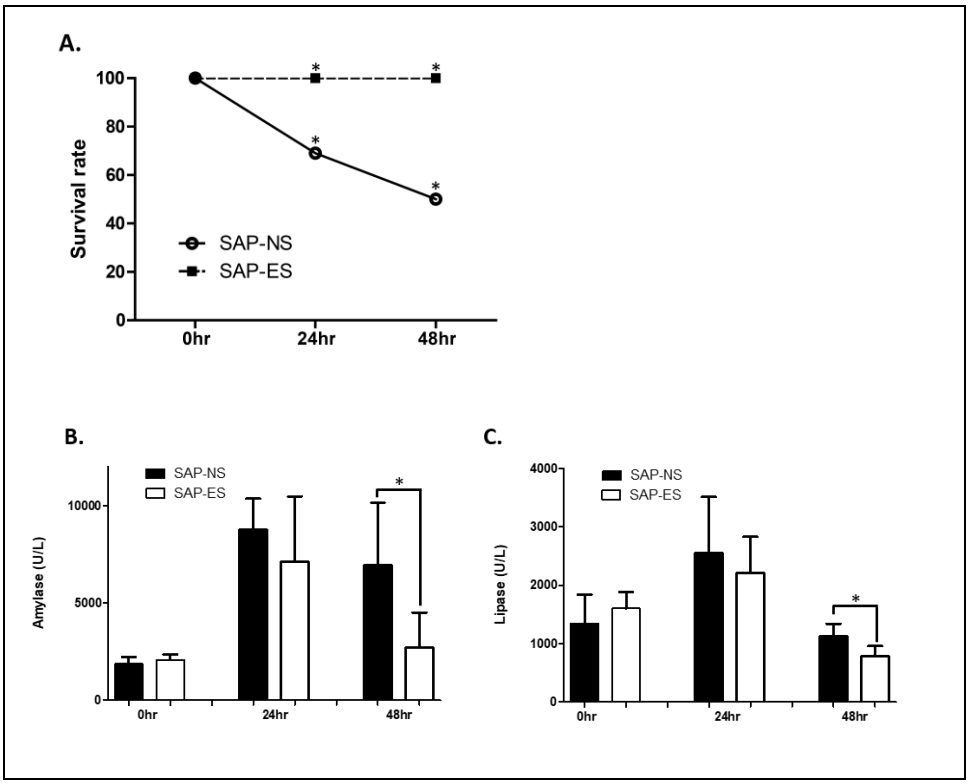

Figure 3. Survival and serum amylase/lipase level of severe acute pancreatitis rat models. (A) Comparison of survival rate of severe acute pancreatitis (SAP) rats after infusion of normal saline (NS) or human embryonic stem cell-derived MSCs (hES-MSC). hES-MSC treatment improved survival of SAP rats significantly, compared to NS. (100% vs. 69.2%, p = 0.025 at 24 h; 100% vs. 50%, p = 0.013 at 48 h) **(B-C)** Comparison of serum amylase and lipase levels of SAP rats after infusion of NS or hES-MSC. hES-MSC treatment significantly reduced serum amylase and lipase levels of SAP rats 48 h after SAP induction, compared to NS. (6930 vs. 2708 U/L, p = 0.022 and 1126 vs. 786 U/L, p = 0.049, respectively). Asterisk (*) indicate statistical significances at $p < 0.05$.

4. Histologic findings and SAP scoring

The histology of SAP rats was evaluated according to a pathologic scoring system previously reported.[13,14,25] Representative histologic findings of the control and SAP groups are presented in Figure 4A. In both groups, severe diffuse necrosis, parenchymal edema, and inflammatory cell infiltration of the pancreas were noted at 24 h and 48 h after SAP induction. One week after the SAP induction, the pancreas exhibited regeneration changes such as newly developed ducts and premature acinar (Figure 4A).

Pancreatic histology from SAP rats received hES-MSC showed decreased interstitial edema and improved parenchymal necrosis compared to SAP rats received NS. (Figure 4B). hES-MSC treatment ameliorated pancreatic edema ($p = 0.006$ at 24 h, $p < 0.001$ at 48 h) and necrosis ($p = 0.034$ at 48 h) significantly, compared to NS treatment (Figure 4B, Table 3). SAP-NS rats presented more separated individual acini and diffuse parenchymal necrosis pattern, while SAP-ES rate presented mainly interlobular or intralobular septa expansion and periductal to focal necrosis, respectively. Number of infiltration of inflammatory cells were not different between groups. SAP-ES rats presented significantly lower total score of SAP than SAP-NS rats ($p = 0.019$ at 24 h, $p < 0.001$ at 48 h), which means hES-MSC infusion may improve pancreatic damage of SAP than NS infusion.

These results confirmed that that SAP was well induced in the rat models in aspect of histopathology and hES-MSC treatment may improve histologic injury of SAP in a rat model.

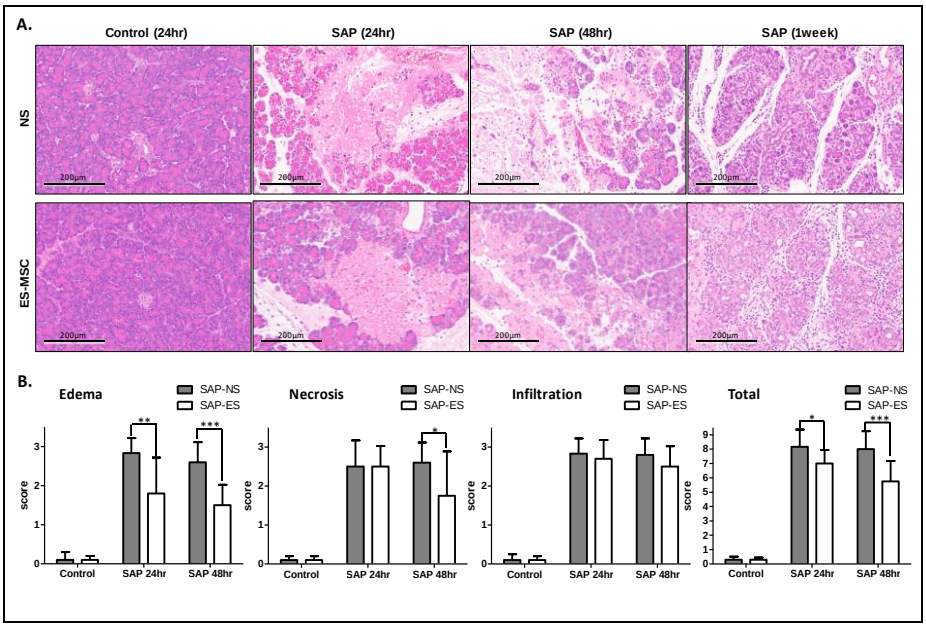

Figure 4. Pathologic results of human embryonic stem cell-derived mesenchymal stem cell injection in a severe acute pancreatitis rat model. **(A)** Representative histologic findings of control and severe acute pancreatitis (SAP) groups at various time points according to NS or of human embryonic stem cell-derived mesenchymal stem cell (hES-MSC) infusion. Scale bars are equal to 200 μm in all images (H&E, ×200). SAP group showed diffuse necrosis and parenchymal edema of the pancreas at 24 h and 48 h. hES-MSC infused rats exhibited reduced parenchymal edema and the pancreas exhibited regeneration changes a week after the SAP induction. (**B)** Pathologic scores in the control and SAP groups at 24 h and 48 h after SAP induction the procedure. hES-MSC infused SAP rats had significantly decreased pancreatic edema scores and total scores. Asterisks (*, **, and ***) indicate statistical significances at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Table 3. Comparison of pathologic scores between SAP-NS and SAP-ES rats

Score (SD)	*hour*	*SAP-NS*	*SAP-ES*	*P value*
Edema	24	2.8 (0.4)	1.8 (0.9)	0.006**
	48	2.6 (0.5)	1.5 (0.5)	< 0.001***
Necrosis	24	2.5 (0.7)	2.5 (0.5)	1.000
	48	2.6 (0.5)	1.5 (0.5)	0.034*
Infiltration of inflammatory cells	24	2.8 (0.4)	2.7 (0.5)	0.491
	48	2.8 (0.4)	2.5 (0.5)	0.152
Total score	24	8.2 (1.2)	7.0 (0.9)	0.019*
	48	8.0 (1.2)	5.8 (1.4)	< 0.001***

Abbreviation : SD; standard deviation, SAP-NS; Severe acute pancreatitis-normal saline injected group, SAP-ES; Severe acute pancreatitis-human embryonic stem cell derived mesenchymal stem cell injected group.

5. Tracking the distribution of hES-MSC by immunofluorescence

Immunofluorescence staining of anti-Human Nuclear Antigen-antibody was performed in hES-MSCs to evaluate whether hES-MSC was migrated to the damaged pancreas (Figure 5A). The distribution of hES-MSCs infused into the SAP rats was traced in the pancreas, liver, and lungs at 24 h, 48 h, and 1 week after hES-MSC infusion (Figure 5B-D). There was no evidence of anti-Human Nuclear Antigen-antibody for hES-MSCs in the pancreas, at any time point (Figure 5B). However, PDX1, the progenitor marker of pancreatic acinar regeneration, was detected at all-time points. This suggested that the regenerated pancreatic tissue after SAP induction was not from the infused human cells, but rather from the rat cells. In the liver, there was no presence of hES-MSCs at any time point (Figure 5C). Interestingly, signals of anti-Human Nuclear Antigen-antibody of hES-MSCs were detected in the lung (Figure 5D). The anti-Human Nuclear Antigen-antibody signal was prominently observed in the lung at 24 h and 48 h. However, there was no distinct signal observed in the lung at 1 week.

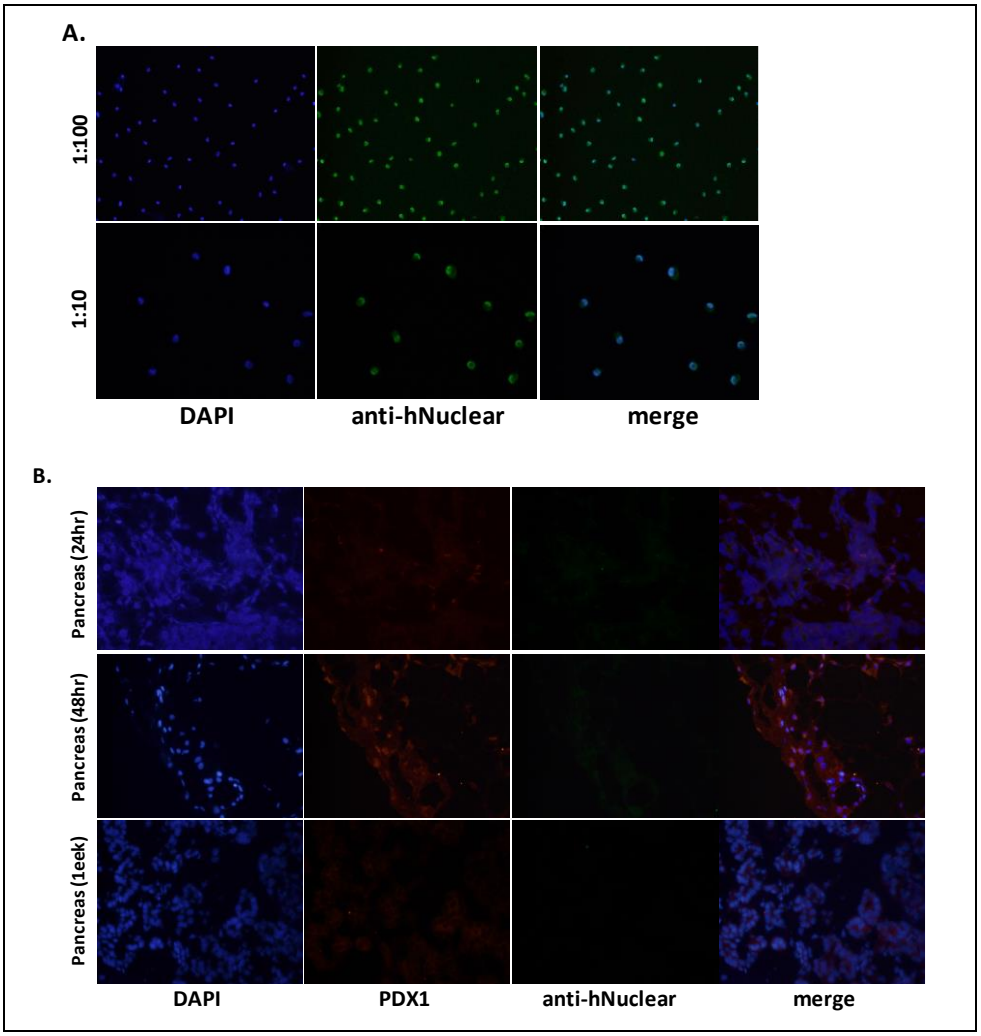

Figure 5. Tracking the distribution of human embryonic stem cell-derived mesenchymal stem cell infused into severe acute pancreatitis rats by tissue immunofluorescence staining. (A) Immunofluorescence stain of human embryonic stem cell-derived mesenchymal stem cell (hES-MSC) before infusion to severe acute pancreatitis (SAP) rat model. **(B)** Immunofluorescence stain of pancreas sections with anti-Human Nuclear Antigen-antibody and anti-PDX1-antibody.

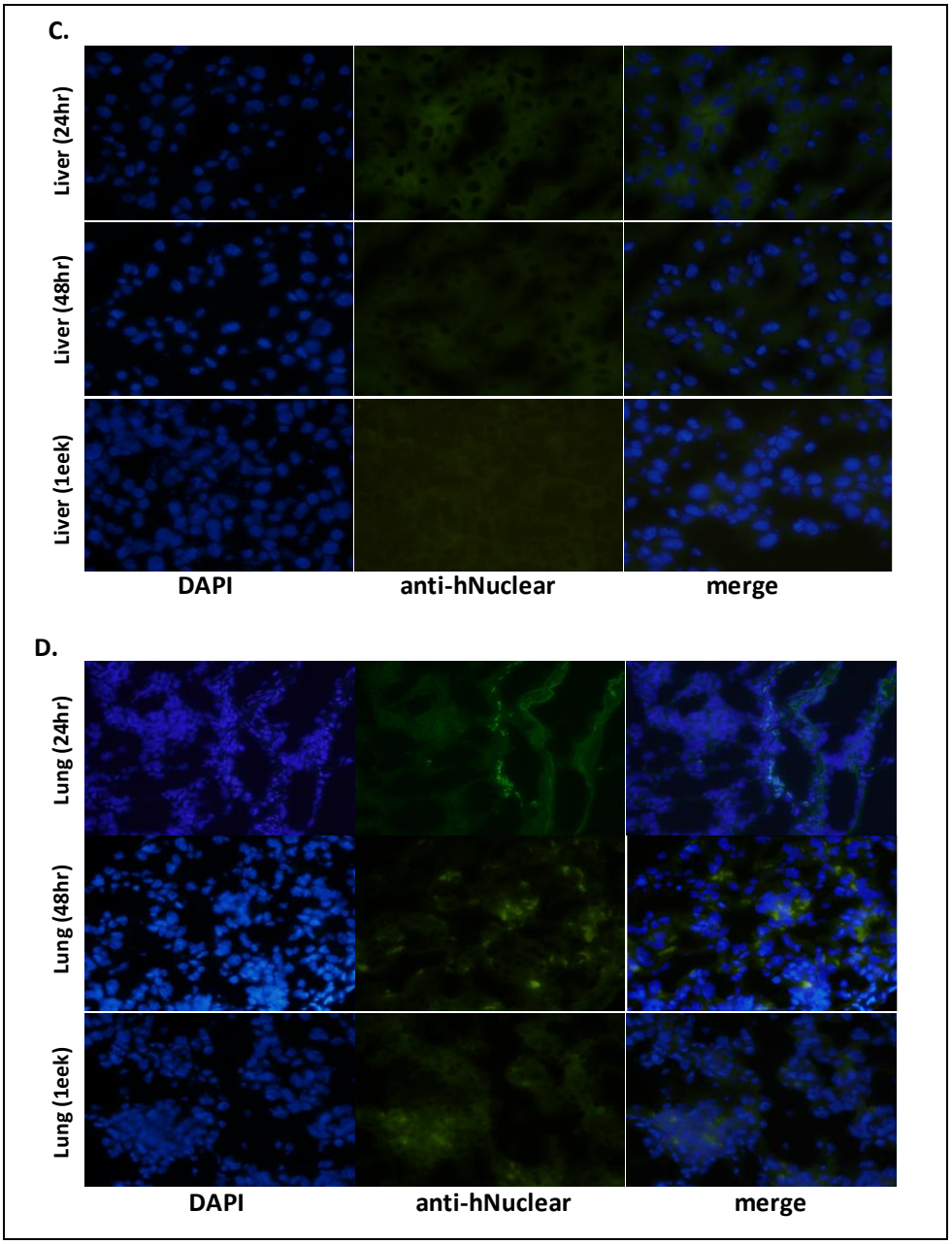

Figure 5. (Continued) (C) Immunofluorescence stain of liver sections with anti-Human Nuclear Antigen-antibody. **(D)** Immunofluorescence stain of lung sections with anti-Human Nuclear Antigen-antibody. (Original magnification, ×400)

6. Tracking the distribution of luciferase transfected hES-MSC with *in vivo* imaging.

Based on immunofluorescent results, we tried to find distribution and excretion of hES-MSCs in rats by live tracing infused cells. Luciferase transfected hES-MSCs (hES-MSC-Luc) maintained their properties *in vitro*, compared to the original hES-MSCs. hES-MSC-Lucs and hES-MSCs had identical microscopic morphological features and cell size under the microscope (Supplementary Figure S1A, B). Expression of CD34 (-), CD45 (-), CD73 (+), and CD105(+) were not different between hES-MSC and hES-MSC-Luc (Supplementary Figure S1C). *in vivo* linearity of hES-MSC-Luc presented in IVIS spectrum CT system. When subcutaneously administered to the 6 points of dorsal skin in normal mice (starting cells $1x10^5$ cells and serially diluted to 6.25 x 10^3 cells), the injected amount of hES-MSC-Luc and the gross intensity of the captured bioluminescence intensity increased proportionally (Supplementary Figure S1D).

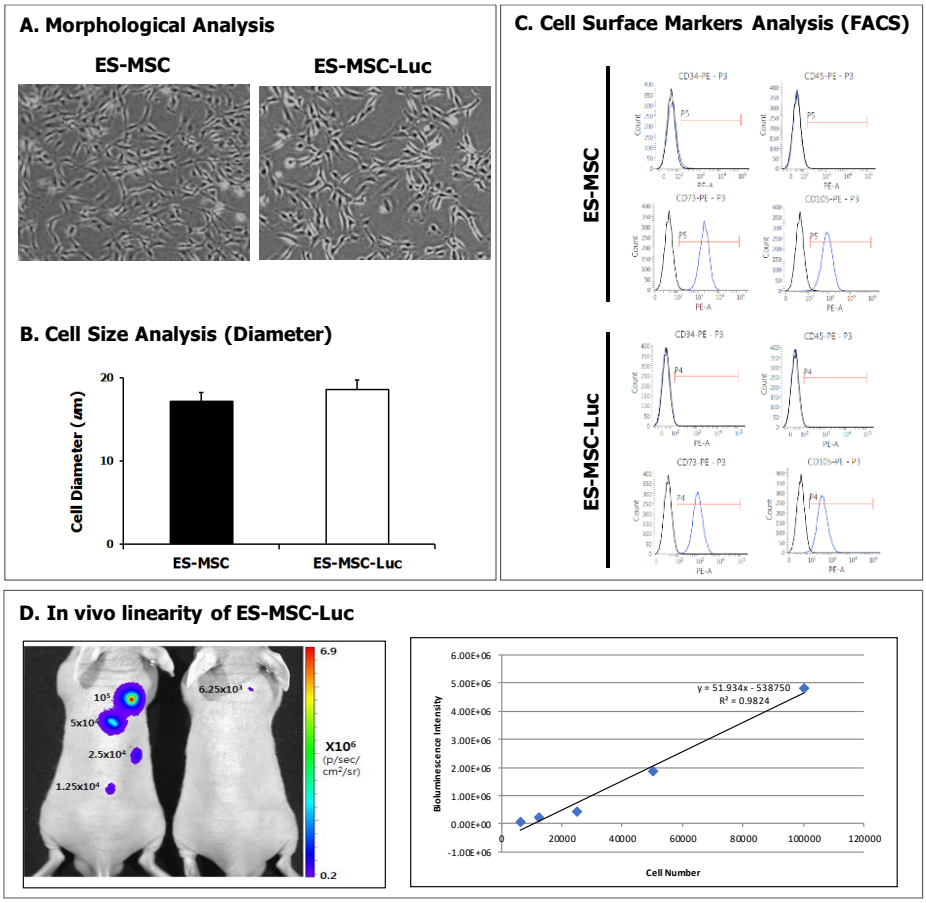

Figure S1. Characteristics of luciferase transfected human embryonic stem cell-derived mesenchymal stem cell. (A, B) Luciferase transfected human embryonic stem cell-derived mesenchymal stem cell (hES-MSC-Luc) and hES-MSC presented identical microscopic morphological features and cell size. **(C)** Cell surface markers CD34 (-), CD45 (-), CD73 (+) and CD105(+), were not different between hES-MSC and hES-MSC-Luc. **(D)** *in vivo* linearity of hES-MSC-Luc presented in IVIS spectrum CT system. The injected amount of hES-MSC-Luc and the gross intensity of the captured bioluminescence intensity increased proportionally when subcutaneously administered to the 6points of dorsal skin in normal mice.

Luciferase transfected hES-MSCs (hES-MSC-Luc) were infused to control and SAP rats via the tail vein. The gross intensity of the captured fluorescence signal indicated that in control rats, infused hES-MSC-Luc spread in the lungs immediately and washed out of the entire body within 24 h of injection (Figure 6A). In SAP rats, infused hES-MSC-Luc spread in the lungs immediately and remained in the lungs for more than 24 h. However, there was no apparent signal in the pancreas. All signals were washed out of the entire body by 72 h after injection (Figure 6B). The fluorescence signal was measured in the lungs, liver, and pancreas, which were harvested immediately after obtaining gross intensity images using the Caliper IVIS Lumina II *in vivo* imaging system (Caliper Life Sciences, Hopkinton, MA, US). Results indicated no definite signal in the liver and pancreas of the control rat 24 h after hES-MSC-Luc injection, and a weak signal in the lungs (Figure 6C). In SAP rats, there was no definite signal in the liver or pancreas 24 h after hES-MSC-Luc injection, however, intense signaling was observed in the lungs (Figure 6D). There was no definite signal in the harvested organs of SAP rats, and all hES-MSC-Luc were washed out of the entire body by 72 h after hES-MSC-Luc injection (Figure 6E). These results suggested hES-MSCs intravenously infused into the SAP rat model did not accumulate in the injured pancreas, but rather in the lungs temporarily, and then they were thoroughly washed out about 72 h after infusion.

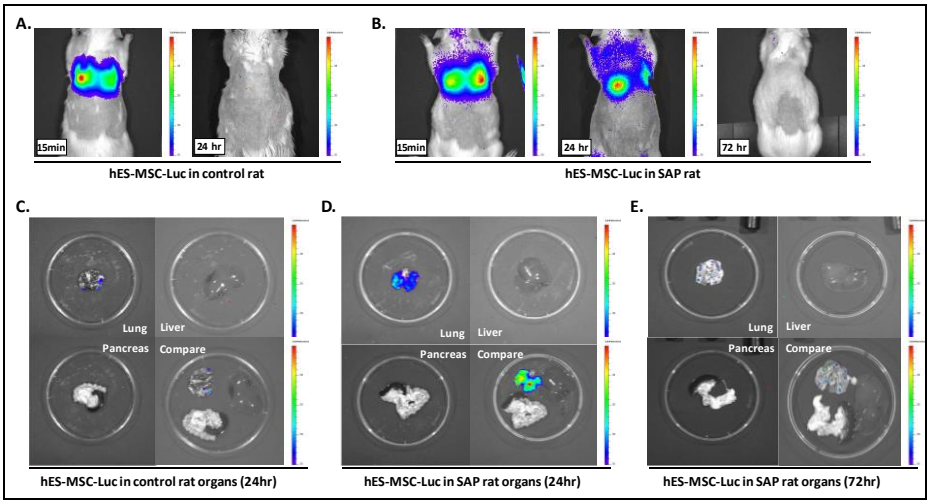

Figure 6. Tracking the distribution of luciferase transfected human embryonic stem cell-derived mesenchymal stem cell (hES-MSC-Luc) infused into a rat *in vivo*. (A) *In vivo* imaging of the control rat shows that infused luciferase transfected hES-MSC-Luc spread in the lungs immediately after infusion and washed out within 24 h of the injection. (**B)** *In vivo* imaging of the SAP rat shows that infused hES-MSC-Luc spread in the lungs immediately after infusion and remained in the lungs for more than 24 h. All signals were washed out 72 h after injection. **(C)** Harvested lungs, liver and pancreas of control rats at 24 h after hES-MSC-Luc injection. There was no definite signal in the liver or pancreas except a weak signal observed in the lungs. (**D)** Harvested lungs, liver and pancreas of SAP rat at 24 h after hES-MSC-Luc injection. Intense fluorescent signal was detected in the lung, but not in the liver and the pancreas. (**E)** Harvested lungs, liver and pancreas of SAP rat at 72 h after hES-MSC-Luc injection. There was no definite signal in the lungs, liver and pancreas

7. Determination of serum inflammatory cytokines expression

To evaluate the immunomodulatory function of hES-MSC, we analyzed the expression of immune modulators after ES-MSC administration by ELISA (Figure 7A-E). Normal (NL) presented baseline level of cytokines in the normal rat serum. Normal levels of cytokines were calculated by pooling the values of all subgroups at 0 h. All detailed values of cytokines were presented in Table 4. Serum levels of IL-1β, IL-6, INF-γ, and TNF-α in SAP rats were significantly elevated at 24 h after the SAP induction, compared to control (Ctrl) rats. When comparing the effect of NS and hES-MCS treatments in SAP rats, IL-1β and IL-6 were significantly reduced at 48 h in the SAP-ES group, compared to the SAP-NS group (43.83 vs. 58.67 pg/mL, $p = 0.002$; and 199.6 vs. 221.8 pg/mL, $p = 0.002$, respectively. Figure 7A, B). SAP-ES rats showed significantly reduced INF- γ and TNF- α compared to SAP-NS rats at 24 hours (80.67 vs. 116.8 pg/mL, $p = 0.0005$; and 43.15 vs. 64.43 pg/mL, $p = 0.047$, respectively) and 48 hours (81.13 vs. 108.3 pg/mL, $p < 0.0001$; and 43.75 vs. 70.17 pg/mL, $p = 0.0007$, respectively), (Figure 7C, D). TGF- β was not significantly different between SAP-NS and ES groups (Figure 7E). Serum inflammatory cytokines expression analysis results indicate the mechanism of hES-MCSs may be related to a reduction of damage to the pancreatitis and may be associated with the immunomodulatory function of MSCs.

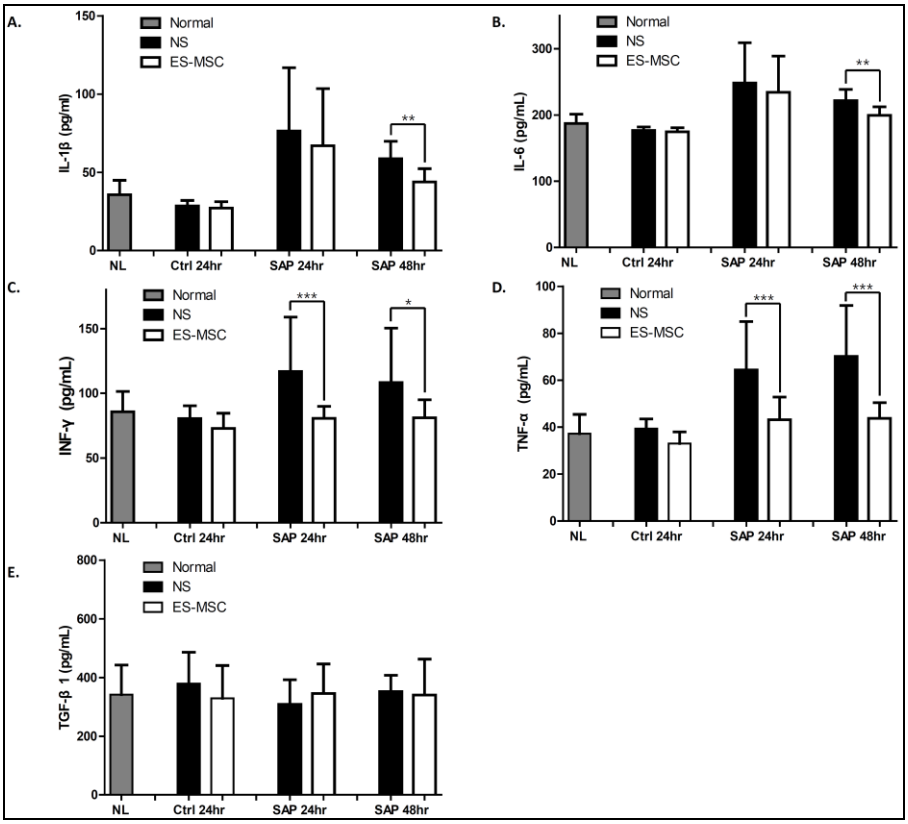

Figure 7. Serum inflammatory cytokines expression after infusion of human embryonic stem cell-derived mesenchymal stem cell. Enzyme-linked immunosorbent assay revealed effects of human embryonic stem cell-derived mesenchymal stem cell (hES-MSC) treatment on the expression levels of IL-1β, IL-6, INF-γ, TNF-α, and TGF-β. Normal (NL) presented baseline level of cytokines in the normal rat serum. Normal levels of cytokines were calculated by pooling the values of all subgroups at 0 h. Control (Ctrl) means rats did not receive severe acute pancreatitis (SAP) induction and SAP means rats received SAP induction. NS and ES-MSC mean normal saline (NS) and hES-MSC infusion for control or SAP rat models. **(A-B)** IL-1β and IL-6 were significantly reduced at 48 h in the SAP-ES group, compared to the SAP-NS group (43.83 vs. 58.67 pg/mL, $p = 0.002$; and 199.6 vs. 221.8 pg/mL, $p = 0.002$, respectively. **(C-D)** SAP-ES rats showed significantly reduced INF- γ and TNF- α compared to SAP-NS rats at 24 hours (80.67 vs. 116.8 pg/mL, $p = 0.0005$; and 43.15 vs. 64.43 pg/mL, $p = 0.047$, respectively) and 48 hours (81.13 vs. 108.3 pg/mL, $p < 0.0001$; and 43.75 vs. 70.17 pg/mL, $p = 0.0007$, respectively). **(E)** TGF- β was not significantly different between SAP-NS and ES groups. In hES-MSC infused SAP rats, IL-1β, IL-6, INF-γ, and TNF-α were significantly reduced, compared to NS infused SAP rats. Asterisks (*, **, and ***) indicate statistical significances at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

Table 4. Comparison of serum inflammatory cytokines expression after infusion of human embryonic stem cell-derived mesenchymal stem cell.

	Cytokine	*Normal (0hr)*†	*24hr*	*48hr*
Control-NS, Mean, pg/mL (SEM)	**IL-1β**	35.62 (0.91)	28.45 (1.13)	N/A
	IL-6	187.3 (1.37)	176.5 (1.69)	N/A
	IFN-γ	85.7 (1.61)	80.56 (3.12)	N/A
	TNF-α	37.25 (0.83)	39.37 (1.31)	N/A
	TGF-β1	341.6 (9.89)	378.9 (33.89)	N/A
Control-ES, Mean, pg/mL (SEM)	**IL-1β**	35.62 (0.91)	27.17 (1.44)	N/A
	IL-6	187.3 (1.37)	174.6 (2.15)	N/A
	IFN-γ	85.7 (1.61)	72.89 (4.15)	N/A
	TNF-α	37.25 (0.83)	32.98 (2.49)	N/A
	TGF-β1	341.6 (9.89)	384.4 (6.635)	N/A
SAP-NS, Mean, pg/mL (SEM)	**IL-1β**	35.62 (0.91)	76.33 (7.96)	58.67 (3.52) **
	IL-6	187.3 (1.37)	248.3 (11.93)	221.8 (5.28) **
	IFN-γ	85.7 (1.61)	116.8 (8.26) ***	108.3 (13.29) *
	TNF-α	37.25 (0.83)	64.43 (3.89) ***	70.17 (6.85) ***
	TGF-β1	341.6 (9.89)	308.6 (16.50)	351.9 (17.50)
SAP-ES, Mean, pg/mL (SEM)	**IL-1β**	35.62 (0.91)	66.94 (7.79)	43.83 (2.46)**
	IL-6	187.3 (1.37)	234.2 (11.69)	199.6 (3.69)**
	IFN-γ	85.7 (1.61)	80.67 (2.07) ***	81.13 (4.00)*
	TNF-α	37.25 (0.83)	43.15 (2.16) ***	43.75 (1.93) ***
	TGF-β1	341.6 (9.89)	345.4 (22.57)	340.5 (35.21)

† Normal levels of cytokines were calculated by pooling the values of all subgroups at 0 h.

Asterisks (*, **, and ***) indicate statistical significances at $p < 0.05$, $p < 0.01$ and $p < 0.001$, compared between SAP-NS and SAP-ES groups.

Abbreviation : SEM; standard error of the mean, SAP-NS; Severe acute pancreatitis-normal saline injected group, SAP-ES; Severe acute pancreatitis-human embryonic stem cell derived mesenchymal stem cell injected group, N/A; not available.

IV. DISCUSSION

In this study, we present hES-MSC can be a therapeutic agent for SAP in rat model. hES-MSC treatment improved survival rates and reduced the serum pancreatic enzyme levels in SAP-induced rats. Pathologic severity was also ameliorated in SAP rats after hES-MSC infusion. These results suggest that hES-MSC may be an effective treatment for SAP.

Despite the high mortality of SAP, conservative treatment measures remain the only therapeutic approach. It is currently thought that SAP develops from pancreatic acinar cell damage due to external stressors. Autodigested pancreatic enzymes leaked from damaged acinar cells trigger a local inflammatory processes. This process consequently accelerates the break-down of pancreatic acinar cells and leads to a vicious cycle which can causes systemic inflammatory response syndrome (SIRS) and multi-organ failure. Treatment to break this cycle would be of great help to patients with SAP. MSC-treatment is expected to improve SAP through the ability of the pluripotent cells to differentiate to the damaged organ cells and their immunomodulatory function which can block inflammatory cycles.[26] Various animal studies have been conducted with promising results.[13,14,27,28] However, to date no MSC-based advanced therapy medicinal products (ATMPs) for SAP have been reported. This is because the mechanism of MSC for the treatment of SAP is not yet clear. It is also due to the diversity of MSCs derived from different sources and the varying effectiveness of MSC-treatment, owing to donor variability.[22]

In our study, the immunomodulating function of hES-MSCs was verified by

the time-phase reduction of inflammatory cytokines after hES-MSC administration. These results indicate that hES-MSCs have a therapeutic effect on SAP via immunomodulation. The immunomodulatory effect was indirectly observed in our study by the decreased level of serum cytokine expression with attenuation of pathologic damage of SAP. Previous studies reported that pro-inflammatory cytokines are associated with the pathogenesis of SAP.[29] IL-1β, IL-6, and TNF-α are the main pro-inflammatory cytokines which are released by innate immune cells during SAP.[30-32] In addition, INF- γ has been reported to increase in various inflammatory diseases.[33,34] It has also been reported to increase in SAP by T helper type 1 (Th1) cells, which increased during SAP.[13] One study reported that the severity of pancreatitis can be attenuated by blockade of IL-6 *in vivo*.[35] In the current study, IL-1β, IL-6, INF-γ, and TNF-α were significantly reduced after hES-MSC injection in SAP rats, in concordance with previous animal studies.[13,27,36] Further studies on the signaling pathways and inflammatory cells associated with pro-inflammatory cytokine changes are necessary to elucidate the specific mechanism of action. However, the current results suggest that hES-MSCs improves SAP through an immunomodulating function.

Studies on the administration of MSCs to SAP have been carried out using various animal models, and stem cells from diversiform origin. The overall therapeutic effect of MSC treatment has been recognized, still there are conflicting suggestions regarding the mechanisms of MSCs' effect, and methods

for identifying the mechanism differ between studies. Jung et al. investigated the effect of MSCs derived from bone marrow after induction of SAP in rats, and reported a significant survival increase and improved pathologic findings, compared to a control group. In addition, CM-DiI (CM-1,1-dioctadecyl-3,3,3-tetramethylindo-carbocyanine perchloride) tagging was used to trace MSCs and showed homing of MSCs to the damaged pancreas.[13] Yang et al. reported that the effect of MCSs derived from human umbilical cord was expressed in a dose-dependent and time-dependent manner.[14] Tu and Hua reaffirmed that MSCs from rat bone marrow homed to damaged pancreas through CM-DiI tagged MSCs, in a rat model.[37,38] These studies concluded that the recovery of pancreatic injury by MSC treatment was due to the migration and implantation of MSCs to the pancreas and their immunomodulatory effect which reduced the inflammatory process of pancreatitis. However, of the two mechanisms, migration and implantation of MSCs are still controversial. Yin et al. studied MSCs derived from rat bone marrow with CM-DiI tagging in a rat pancreatitis model, but found no homing effect of MSCs to the damaged pancreas.[39] He et al. reported similar results—that the CM-DiI tagging method indicated that MSCs did not move to the damaged pancreas. They also traced MSCs by luciferase transfection in a mouse model and observed same results.[27] Yin et al. reported that extracellular vesicles derived from MSCs demonstrated a therapeutic effect on pancreatitis in a rat model.[40] However, several other publications from a similar period reported that the homing effect of MSC was

still under investigation.[41,42] Zhao et al. confirmed the homing effect of MSCs by tracing MSCs from a male donor in female recipients.[43] Although various experimental studies suggested possible mechanisms of MSCs related to pancreatitis amelioration, the exact mechanism remains controversial. The results of studies about MSC engraft in pancreatitis animal models are summarized in Table 5.

Table 5. Summary of previous studies about MSC engrafment in pancreatitis models

Author	MSC source	Dose	Animal	SAP induction	MSC tracing	MSC engraftment	Ref.
This study	**Human ES**	**5*10^6**	**Rat**	**3% TC**	**H-Nuclear Antigen /Luc-MSCs**	**No evidence**	
Jung	Human BM	1x10^6	Rat	Cerulein /3% TC	CM-DiI /hCEN	Possible	[13]
Yang	Human UC	5x10^6	Rat	3% TC	N/A	N/A	[14]
Meng	Human UC	1x10^7	Rat	3% TC	N/A	N/A	[28]
Tu	Rat BM	2x10^7	Rat	4% TC	CM-DiI	Possible	[38]
Hua	Human UC	2x10^6	Rat	3% TC	CM-DiI	Possible	[37]
Jung	Human BM	1x10^6	Rat	Cerulein + LPS	CM-DiI	Possible	[41]
Qian	BM	1x10^7	Rat	3% TC	N/A	N/A	[36]
Yin	Rat BM	1x10^6	Rat	L-Arg	CM-DiI	No evidence	[39]
He	Human BM	2x10^6	Mouse	2% TC	Luc-MSCs /CM-DiI	No evidence	[27]
Kawakubo	Rat FM	1x10^6	Rat	4% TC	N/A	N/A	[44]
Kim	Canine AT	1x10^7	Rat	3% TC	CM-DiI	Possible	[42]
Yin	Rat BM	MVs	Rat	4% TC	N/A	N/A	[40]
Zhao	Rat BM	5-7x10^7	Rat	3% TC	male MSC PCR	Possible	[43]
Lu	Rat BM	1x10^6	Rat	5% TC	CM-DiI	No evidence	[45]

Abbreviation : MSC; mesenchymal stem cell, SAP; severe acute pancreatitis, BM; bone marrow, US; umbilical cord, AT; adipose tissue , ES; embryonic stem cell, TC; taurocholate, LPS; lipopolysaccharide, L-arg; L-arginine, CM-DiI; CM-1,1-dioctadecyl-3,3,3-tetramethylindo-carbocyanine perchloride, hCEN; human centromere, Luc-MSC; luciferase transfected MSC, PCR; polymerase chain reaction, N/A; not available.

There has been controversy over the migration of MSCs to the damaged pancreas. Microscopic tracing by Immunofluorescence study and *in vivo* cell tracking by luciferase transfection in this study did not show that infused hES-MSC was engrafted to damaged pancreas. It is meaningful that in this study, live tracing of MSCs in vivo using an IVIS spectrum CT system in a rat model was performed for the first time, although our results may not lend us to draw conclusions about inconsistencies in existing previous studies. Our results suggest that the therapeutic mechanism of hES-MSC is less likely to be engraftment to damaged organs, especially the pancreas.

Another important issue for developing MSC-based therapies is whether MSCs remains in the living body and which has the potential to causes neoplastic disease.[46] There are concerns about the risks of tumor formation, especially in human embryonic stem cells.[47,48] In contrast to embryonic stem cells, human MSCs are considered have immune privilege and are relatively free from the risk of tumorigenicity.[49] It was considered important to investigate whether hES-MSC, a kind of MSCs with properties of embryonic stem cells, remains in the body after cell therapy. In this study, although hES-MSCs were present in the body of SAP rats longer than in the controls, hES-MSCs were shown to be totally removed by 72 hours post infusion. In the current study, few hES-MSC-Luc were detected at 72 hours *in vivo* on tracing images in SAP rats, and there was no observed expression of Human Nuclear Antigen in the immunofluorescence at 1 week. These results suggest that residual neoplastic

disease is unlikely to be caused by hES-MSC treatment.

An interesting phenomenon which we observed in the current study was that the hES-MSCs were accumulated in the lungs, rather than the damaged pancreas; and stayed longer in the lungs of SAP rats than control rats. One previous study tried to traced MCSs after infusion to a SAP mouse model, using luciferase transfected MSCs *in vivo*.[27] The study reported that there was no evidence of MSCs homing to the damaged pancreas; instead, MSCs were primarily accumulated in the lungs 24 h after injection. These results were similar to ours, however, we traced luciferase transfected MSCs in a SAP rat model, with a control group. We observed that the infused MSCs were deposited in the lungs and then exited the body over time, with a longer wash-out time in SAP rats compared to the control. The primary deposition of MSCs in the lung is 'passive homing' which may be due to the average diameter of the MSC (diameter 15-30 μm) being bigger than that of the alveolar-capillary (diameter 8 μm).[50] This phenomenon is termed the 'pulmonary fist-pass effect.[51-53] Entrapped MSCs are thought to be cleared by a function of phagocytic monocytes engulfing apoptotic MSCs[54,55] or by facilitating the passage of larger cells through smaller vessels.[56,57] Moreover, SAP induction in a rat model may be accompanied by lung damage from systemic inflammation,[14,36] this may make it difficult for MSCs to pass through capillaries. In our study, lung damage may have caused more pronounced first-pass effect in the SAP rats. These suggest that the homing-like

phenomenon of MSCs in the previously reported pancreatitis animal model studies was probably caused by mechanical entrapment rather than biological engraftment.

This study has several limitations. First, the exact accurate mechanism of the hES-MSCs exerted by immunomodulation was unclear. We asserted the representative inflammatory cytokines were down-regulated by the hES-MSCs. However, which signaling pathway or and which inflammatory cells are involved in this progress remains unknown. Second, although the hES-MSCs were not observed in the lungs or liver (which are the most prominent organs of the passive homing effect) we did not study all peripheral capillary tissues. Extended long-term survival studies are needed to confirm malignant potential of hES-MSC. Third, the effect of hES-MSC in human pancreatitis may be different. Among various mechanisms of human pancreatitis, only chemical induced pancreatitis was investigated in our study. Therapeutic effect for other organ damages which may associated with SAP has not been confirmed. Moreover, the clinical treatment intervention timing may be different from the study setting, because of the severity of SAP at the time of diagnosis diverse per patient.

V. CONCLUSION

In this study, the effects of hES-MSC therapy were investigated in an induced SAP rat model. Our results showed that hES-MSC therapy was a safe and

efficient treatment which significantly reduced mortality and pancreatic damage in a rat model of SAP. It seems that hES-MSCs modulated inflammation by inhibiting inflammatory cytokines in animal models. Infused hES-MSC did not migrated to the damaged pancreas and washed out the SAP rat by about 72 hours after administration. Further studies regarding immunomodulating mechanisms and long-term effect of MSC are needed.

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ABSTRACT (IN KOREAN)

랫드 모델에서 유도된 중증 급성 췌장염에 대한 인간 배아 줄기 세포 유래 중간엽 줄기세포 투여의 안전성과 효과 검증

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조 중 현

연구배경: 중증 급성 췌장염은 높은 치사율을 보이는 치명적인 질환으로 여겨지고 있지만, 효과적인 치료제는 아직까지 존재하지 않는다. 중간엽 줄기세포는 다분화 능력과 면역조절 기능으로 인하여 중증 급성 췌장염의 가능성 있는 치료제 중 하나로 고려되고 있지만, 인간 배아 줄기 세포 유래 중간엽 줄기 세포의 췌장염 치료제로서의 가능성에 대해서는 아직까지 연구된 바가 없다.

연구방법: Sprague-Dawley 랫드 의 3% sodium taurocholate 담도

내 투여 수술법을 이용하여 중증 급성 췌장염 랫드 모델을 구축하였다. 췌장염 유도 수술을 한 직후에 꼬리 정맥을 통하여 생리식염수 또는 인간 배아 줄기 세포 유래 중간엽 줄기 세포 를 투여하고 그 치료효과를 생리식염수 투여 대조군과 비교하였다.. 수술 후 24, 48 시간 째 생존률 및 혈액 내 췌장 효소 농도 및 염증성 사이토카인 농도를 ELISA 법을 통하여 측정하였고, 24, 48시간 째 생존한 중증 급성 췌장염 rat 모델의 췌장을 병리 분석하여 인간 배아 줄기 세포 유래 중간엽 줄기세포의 치료 효과를 확인하였다. 인간 배아 줄기 세포 유래 중간엽 줄기 세포의 랫드 체내 분포를 확인하기 위하여 anti-Human Nuclear Antigen-antibody 면역 형광 염색 및 Luciferase 가 주입된 인간 배아줄기 세포 유래 중간엽 줄기세포를 이용하여 생체 분포를 추적하였다.

연구결과: 인간 배아줄기 세포 유래 중간엽 줄기 세포를 투여한 중증 급성 췌장염 랫드 모델에서 생리식염수를 투여한 경우에 비하여 통계적으로 유의한 생존률의 향상이 확인 되었고, 혈중 아밀라아제와 리파아제 농도가 유의하게 낮게 측정 되었다. 또한, 인간 배아 줄기 세포 유래 중간엽 줄기세포를 투여한 그룹에서 생리식염수를 투여한 그룹에 비하여 췌장의 병리학적 손상 소견이 유의하게 완화된 것으로 판단되었다. 면역형광 염색 결과 랫드 모델의 폐 조직에 인간 배아 줄기 세포 유래 중간엽 줄기세포가 존재하는 것이 확인 되었으나

랫드 모델의 췌장에서는 발현이 확인되지 않았다. Luciferase 가 주입된 인간 배아줄기 세포 유래 중간엽 줄기세포를 실시간 생체 추적 관찰 하였을 때, 랫드 체내 주입 즉시 폐에 모이는 것으로 관찰 되었고, 정상 랫드에서는 약 24시간 후에 체내에서 배출 되었으나 중증 급성 췌장염 랫드 모델에서는 약 72시간 후에 배출됨을 확인할 수 있었다. 염증성 사이토카인 IL-1β, IL-6, INF-ɣ, 및 TNF-α 의 혈중 농도는 는 인간 배아줄기 세포 유래 중간엽 줄기세포를 투여 한 뒤 생리식염수를 주입한 경우와 비교하여 유의한 감소가 확인되었다.

결론: 인간 배아 줄기 세포 유래 중간엽 줄기 세포 치료는 안전하교 효과적으로 중증 급성 췌장염 랫드 모델의 췌장 손상을 줄이고 사망률을 감소시키는 것으로 확인되었으며, 이는 염증성 사이토카인 억제를 통한 면역 조절 기능으로 인한 것으로 판단되었다. 이는 인간 배아 줄기 세포 유래 중간엽 줄기세포가 중증 급성 췌장염에 대한 치료 수단으로 사용될 수 있음을 시사하는 결과라 하겠으며, 향후 추가 연구가 필요할 것으로 판단된다.

핵심 되는 말: 중증 급성 췌장염, 인간 배아 줄기 세포 유래 중간엽 줄기 세포, 랫드 모델