Mesenchymal stem cells cultured in hypoxic conditions have multi-directional therapeutic effects on mice with liver cirrhosis through prostaglandin E2 and miR210 productione

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INTRODUCTION

Mesenchymal stem cells (MSCs) are easily expanded. They can be acquired from medical waste such as adipose and umbilical cord tissues, are influenced by culturing conditions, and are anti-inflammatory, antioxidant, anti-fibrotic, and angiogenic. We analyzed the multi-directional effects of MSCs cultured in hypoxic conditions and their underlying mechanisms in the treatment of liver cirrhosis model mice.

Since they have multiple functions, readily expand, and are minimally antigenic, both autologous and allogeneic MSCs have been used in >900 clinical trials to treat various diseases. Several basic studies have been conducted using MSCs to treat various liver disease models such as cirrhosis, NASH, and acute injury. To date, ~50 trials used autologous and allogeneic MSCs to target acute and chronic liver diseases.

AIM

In this study, we analyzed the multi-directional effects of MSCs cultured in hypoxic conditions and their underlying mechanisms in the treatment of liver cirrhosis model mice.

METHOD

Human bone marrow-derived MSCs cultured in hypoxic (5% O_2; hypoMSCs) and normal oxygen conditions (21% O_2; norMSCs) were compared by cap analysis of gene expression (CAGE) with or without serum from liver cirrhosis patients. The therapeutic effects of MSCs such as serum liver enzyme induction and fibrosis regression were evaluated by injecting 1×10^6, 2×10^5, or 4×10^4 MSCs / mouse into the tail veins of CCl_4-induced liver cirrhosis model mice. Intravital imaging was performed with a two-photon excitation microscope to confirm the various MSC migration paths to the liver.

RESULTS

Table 1. Information regarding patient profiles.

	sex	age	etiology	Child-Pugh Score	ALT(U/l)	Platelets(× 10000/μl)
LC1	F	80	NASH	B（7)	20	6.1
LC2	M	67	Alcohol	A（5)	22	13.6
LC3	F	54	Alcohol	B（8)	14	5.7

Figure 1

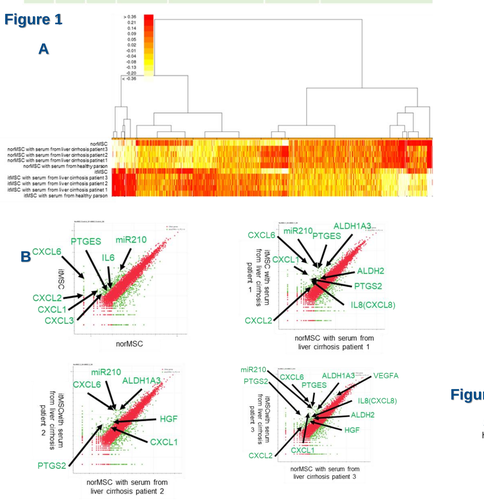

Figure 1. HypoMSC produced more PTGES and miR-210 than norMSC
Cap Analysis Gene Expression (CAGE) of mRNA expression changes in hypoMSC and norMSC with- or without serum from a healthy person or a liver cirrhosis (LC) patient. (*A*) A heat map revealed that the mRNA expression patterns of hypoMSC and norMSC markedly differed in the absence of human serum culture. Their mRNA expression levels evidently changed after human serum was added. Relative differences in mRNA level between the MSC culture with healthy serum and that with LC serum were smaller than those between the MSC culture without human serum and that with human serum. (B) The miR210, Ptges, Il-6, and Cxcl1,2,3 and 6 genes were upregulated by >4× in hypoMSC compared to those in norMSC. After adding normal or LC serum to both MSCs, Aldh, Il-8, and Hgf were upregulated in hypoMSC compared to norMSC.

Figure 2

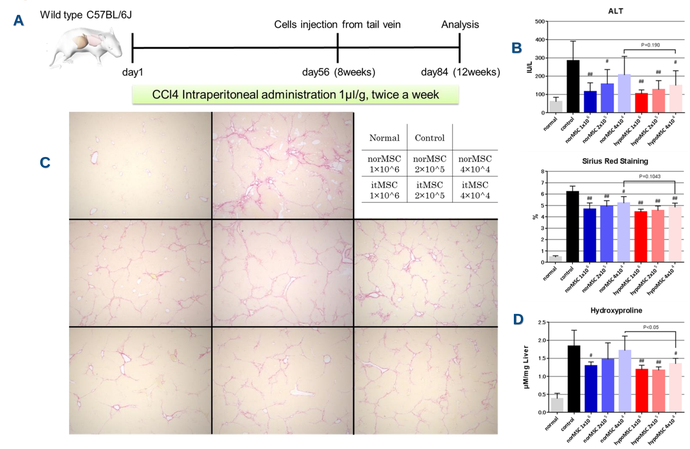

Figure 2. HypoMSC decreased liver damage and fibrosis in mice in a dose-dependent manner
(A) Schematic diagram showing the fibrosis induction, cell administration, and analysis used in the present study. (B) Serum ALT, ALP, total bilirubin, and albumin levels four weeks after cell injection. Data are means ± SD; n = 9–11 mice per group; $P < 0.001$ (ALT, hypoMSC 1×10^6); $P < 0.001$ (ALT, hypoMSC 2×10^5); $P < 0.01$ (ALT, hypoMSC 4×10^4); $P < 0.001$ (ALT, norMSC 1×10^6); $P < 0.01$ (ALT, norMSC 2×10^5) relative to the control.
(C) Sirius red staining and (D) hydroxyproline assay showing that hypoMSC and norMSC mitigated mouse liver fibrosis in a dose-dependent manner, but hypoMSC decreased liver fibrosis more than norMSC. Data are means ± SD; n = 9–11 mice per group; $P < 0.001$ (Sirius red, hypoMSC 1 x 10^6 cells/mice); $P < 0.001$ (Sirius red, hypoMSC 2 x 10^5 cells/mice); $P < 0.001$ (Sirius red, hypoMSC 4 x 10^4 cells/mice); $P < 0.001$ (Sirius red, norMSC 1 x 10^6 cells/mice); $P < 0.001$ (Sirius red, norMSC 2 x 10^5 cells/mice); $P < 0.01$ (Sirius red, norMSC 4 x 10^4 cells/mice) compared to the control. Data are means ± SD; n = 9–11 mice per group; $P < 0.001$ (hydroxyproline, hypoMSC 1 x 10^6 cells/mice); $P < 0.001$ (hydroxyproline, hypoMSC 2x10^5 cells/mice); $P < 0.01$ (hydroxyproline, hypoMSC 4 x 10^4 cells/mice); $P < 0.01$ (hydroxyproline, norMSC 1 x 10^6 cells/mice) relative to the control.

Figure 3

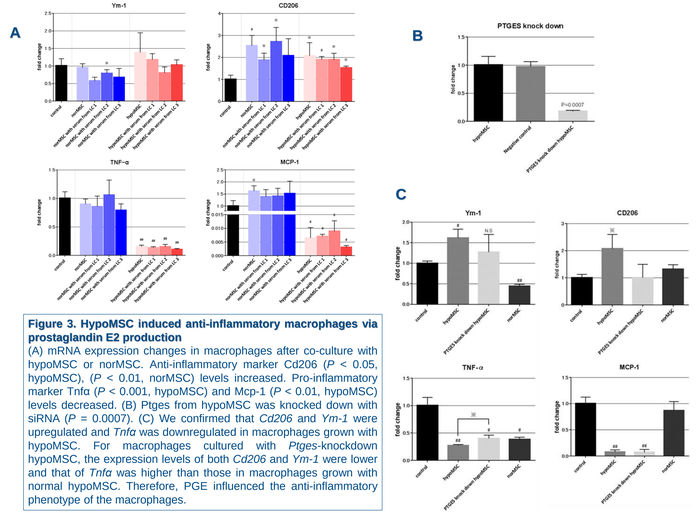

Figure 3. HypoMSC induced anti-inflammatory macrophages via prostaglandin E2 production
(A) mRNA expression changes in macrophages after co-culture with hypoMSC or norMSC. Anti-inflammatory marker Cd206 ($P < 0.05$, hypoMSC), ($P < 0.01$, norMSC) levels increased. Pro-inflammatory marker Tnfα ($P < 0.001$, hypoMSC) and Mcp-1 ($P < 0.01$, hypoMSC) levels decreased. (B) Ptges from hypoMSC was knocked down with siRNA ($P = 0.0007$). (C) We confirmed that *Cd206* and *Ym-1* were upregulated and *Tnfα* was downregulated in macrophages grown with hypoMSC. For macrophages cultured with *Ptges*-knockdown hypoMSC, the expression levels of both *Cd206* and *Ym-1* were lower and that of *Tnfα* was higher than those in macrophages grown with normal hypoMSC. Therefore, PGE influenced the anti-inflammatory phenotype of the macrophages.

Figure 4

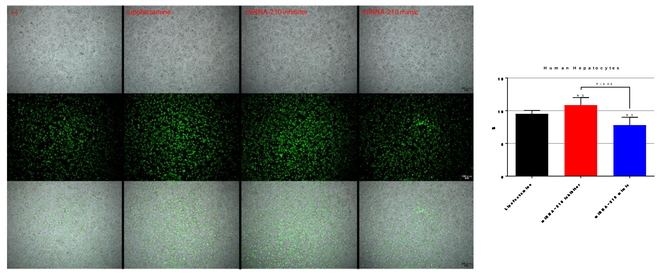

Figure 4. miR-210 reduced hepatocyte apoptosis
(A)(B) Control or miR-210 mimic or miR-210 inhibitor were transfected with PXB-cells (hepatocytes) which are induced apoptosis by actinomycin D. These revealed that apoptosis was significantly reduced in PXB-cells.

Figure 5

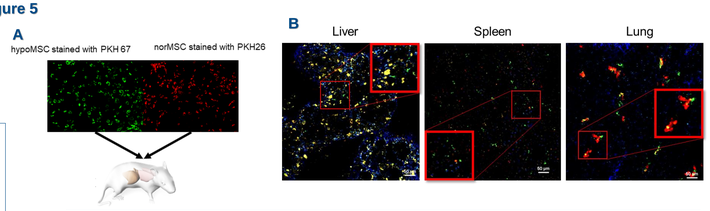

Figure 5. After injection, most norMSC and hypoMSC were trapped in the lung and only a few migrated to the liver
(A) Green cells represent hypoMSCs and red cells represent norMSCs. There were 5 x 10^5 cells of each MSC injected into the cirrhosis mouse model through the tail vein. (B) Intravital imaging was performed on the liver (left panels), spleen (middle panels), and lung (right panels) under a two-photon excitation microscope at 6 h after cell injection. The dense blue fibrous area represents fibrosis. The yellow spots represent hepatocyte debris (scale bar: 50 μm).

CONCLUSIONS

The therapeutic effect of hypoMSCs was mediated by PGE2 and miR210 production etc. and was greater than that of norMSCs. Therefore, MSCs can be manipulated to improve their therapeutic efficacy in the treatment of liver cirrhosis and could potentially serve in effective cell therapy. MSCs produce several factors with multidirectional effects and function as “conducting cells” in liver cirrhosis.

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