

Hypoxia-preconditioned bone marrow mesenchymal stem cells alleviate acute liver failure by regulating the VEGF/c-MET pathway

Mei-Mei Lan

Lanzhou University

Rui-Zhi Shi

Lanzhou University

Zhe-Yu Li

Lanzhou University

Wen-Jun Qiao

First Hospital of Lanzhou University

Yong-Juan Guan

First Hospital of Lanzhou University

Wen-Qiang He

First Hospital of Lanzhou University

Jun-Feng Li

First Hospital of Lanzhou University

Li-Ting Zhang

z1t08@qq.com

First Hospital of Lanzhou University

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Abstract

Background

Acute liver failure (ALF) carries a high mortality with few treatments. Although bone marrow-derived mesenchymal stem cells (BMSCs) hold therapeutic promise, their efficacy is limited by the hostile liver milieu. Hypoxia preconditioning (HP) enhances BMSC adaptability. This study investigated HP-BMSCs for ALF, focusing on the VEGF/c-MET pathway and B-cell immunity.

Methods

Mouse BMSCs were cultured under normoxia (21% O₂) or HP (1% O₂) for 4 hours (h). Western blot and Q-PCR were used to detect the expression levels of HIF-1 α , VEGF and c-Met. ALF model was induced in C57BL/6J mice using D-galactosamine/LPS. Animals were randomized into Control, ALF, ALF + normoxic - BMSC, or ALF + HP-BMSC groups (n = 6). Cells were transplanted via tail vein 4h post-modeling; samples were collected 4h later. Assessments included liver function, cytokines, histology, and molecular/immunological analyses.

Results

HP upregulated HIF-1 α , VEGF, c-MET, and PCNA in BMSCs ($P < 0.01$). In ALF mice, HP-BMSCs outperformed normoxic BMSCs, reducing liver injury, restoring function (ALT, AST, TBIL), and attenuating inflammation ($P < 0.01$). HP-BMSCs activated the hepatic VEGF/c-MET axis (upregulated VEGF, HGF- α , c-Met) and enhanced regeneration ($P < 0.01$). Notably, they modulated intrahepatic B-cells, reducing CD45R+ infiltration while increasing regulatory CD24 + CD38+ subsets.

Conclusions

Our research indicates that HP potentiates BMSCs for ALF primarily via VEGF/c-MET activation, enhancing their proliferative and paracrine capacities. The therapy synergistically promotes regeneration, suppresses inflammation, and reprograms intrahepatic immunity via B-cell modulation.

Introduction

Acute liver failure (ALF) is a critical clinical syndrome triggered by diverse etiologies, including viral infection, drug toxicity, and poisoning. It is characterized by massive hepatocyte necrosis, rapid functional deterioration, and high mortality [1, 2]. While liver transplantation remains the definitive treatment, its widespread application is constrained by donor shortage, surgical risks, immune rejection, and high costs [3]. Hence, developing effective and minimally invasive alternative therapies is an urgent priority.

Stem cell-based regenerative medicine presents a promising avenue. Among various stem cell types, bone marrow-derived mesenchymal stem cells (BMSCs) have attracted significant interest due to their accessibility, ease of expansion, low immunogenicity, and potent paracrine capabilities [4, 5]. Evidence suggests that the therapeutic benefits of BMSCs in liver failure stem not from direct differentiation but primarily from their secretion of bioactive factors (e.g, growth factors, cytokines), which exert anti-apoptotic, anti-inflammatory, pro-angiogenic, and immunomodulatory effects, thereby promoting a reparative microenvironment [6, 7].

However, a major obstacle to their clinical efficacy is the poor survival and functional impairment of transplanted BMSCs within the hostile, ischemic, and inflammatory milieu of the failing liver [8, 9]. Preconditioning strategies, such as hypoxia preconditioning (HP), offer a solution by priming cells to better withstand post-transplantation stress. HP mimics the *in vivo* niche and activates the hypoxia-inducible factor-1 α (HIF-1 α) pathway, which upregulates genes involved in cell survival, homing, and angiogenesis, ultimately enhancing the cells' adaptive and paracrine functions [10–11].

The vascular endothelial growth factor (VEGF) / hepatocyte growth factor (HGF) axis, signaling through the receptor c-MET, is a key regulator of hepatocyte regeneration, vascular remodeling, and tissue repair [12, 13]. We hypothesize that HP may enhance the hepatoprotective effects of BMSCs by activating this VEGF / c-MET pathway. Moreover, while dysregulated immune responses—particularly B lymphocyte-mediated humoral immunity—are increasingly implicated in ALF progression, their modulation by BMSC therapy remains poorly understood.

Therefore, this study aimed to investigate whether hypoxia preconditioning (HP) activates the VEGF / c-MET pathway in mouse BMSCs, to compare the therapeutic efficacy of HP-BMSCs versus normoxia-cultured BMSCs in a mouse model of ALF, and to elucidate the associated mechanisms—with a specific focus on the regulation of intrahepatic reparative factors and the modulation of B lymphocyte-mediated immune inflammation. Our findings are expected to provide novel insights into the mechanisms underlying BMSC therapy and to inform the development of optimized stem cell-based strategies for ALF.

Methods

Cell Culture and Hypoxia Preconditioning

Mouse bone marrow-derived mesenchymal stem cells (BMSCs) were expanded in complete growth medium and maintained at 37°C in a humidified atmosphere containing 5% CO₂. For preconditioning experiments, cells were seeded into 6-well plates at an appropriate density. Upon reaching approximately 40% confluence, the cultures were divided into two experimental groups: a Normoxia group and a Hypoxia Preconditioning (HP) group. The HP group was transferred to a modular hypoxia chamber and incubated for 24 hours under hypoxic conditions (1% O₂, 5% CO₂, balanced with N₂ at 37°C). The Normoxia group was continuously cultured under standard conditions (21% O₂, 5% CO₂ at 37°C) for the

same duration. After the 24-hour treatment period, cells from both groups were harvested for subsequent analysis.

Animal Model and Experimental Groups

An acute liver failure (ALF) model was induced in male C57BL/6J mice by a single intraperitoneal injection of D-galactosamine (800mg/kg) and lipopolysaccharide (100µg/kg) [17]. Following one week of acclimatization, the mice were randomly divided into four groups (n = 6 per group): the Normal Control group received an injection of phosphate-buffered saline (PBS); the ALF Model group received D-GalN/LPS to establish the injury; the Normoxia-BMSC group received D-GalN/LPS followed by a tail vein infusion of 1×10^6 BMSCs cultured under normoxic conditions; and the Hypoxia-BMSC group received D-GalN/LPS followed by infusion of an equal number of BMSCs that had been preconditioned under hypoxia (1% O₂ for 24 hours). All cell transplantations were performed 4 hours after model induction. Four hours after LPS/GalN injection, mice were euthanased, and serum and liver samples were collected to assess the extent of liver injury. Biochemical parameters of serum were evaluated. Liver samples were subjected to histochemical and Western blot analysis for assessment.

Western Blot Analysis

Proteins were extracted from cells and liver tissues using RIPA lysis buffer containing protease and phosphatase inhibitors. The lysates were centrifuged at 12000g for 15 min at 4°C, and the supernatant protein concentration was quantified with a BCA assay. Equal amounts of protein were separated by SDS-PAGE and subsequently transferred onto PVDF membranes. After blocking with 5% non-fat milk, the membranes were incubated overnight at 4°C with specific primary antibodies. Following thorough washing, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1h at room temperature. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection system and quantitatively analyzed with ImageJ software.

Quantitative Real-Time PCR (RT-qPCR)

Total RNA was extracted from cells and liver tissues using TRIzol reagent, and its concentration and purity were assessed spectrophotometrically. cDNA was synthesized from equal amounts of RNA using a reverse transcription kit. Quantitative PCR was performed using a SYBR Green master mix on a real-time PCR detection system, with GAPDH serving as the endogenous control. The primer sequences used are listed in Table 1. The thermal cycling protocol consisted of an initial denaturation at 95°C for 10 seconds, followed by 45 cycles of denaturation at 95°C for 5 seconds and annealing/extension at 60°C for 30 seconds. The relative mRNA expression levels of target genes were calculated using the $2^{-\Delta\Delta Ct}$ method. All reactions were performed in triplicate.

Biochemical Assays

Blood samples collected via the retro-orbital sinus were centrifuged at 1200 g for 15 min at 4°C to separate serum. The serum levels of alanine aminotransferase (ALT) and total bilirubin (TBIL) were measured using a commercial assay kit or determined by an authorized commercial laboratory (Beijing Vital River Laboratory Animal Technology Co., Ltd.).

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum levels of IL-6 and IL-10 were measured using specific commercial ELISA kits according to the manufacturer's protocols. The absorbance was read at 450 nm, and cytokine concentrations were calculated based on the respective standard curves.

Hematoxylin and Eosin (H&E) Staining

Liver tissues were fixed in 4% paraformaldehyde, dehydrated through a graded ethanol series, embedded in paraffin, and sectioned at 4µm thickness. Following deparaffinization and rehydration, the sections were stained with hematoxylin and eosin using a standard protocol. After dehydration and clearing, the sections were mounted with neutral resin and examined under a light microscope. Histopathological changes, including hepatocyte necrosis and inflammatory cell infiltration, were assessed.

Immunohistochemistry (IHC)

Paraffin-embedded liver sections were deparaffinized, rehydrated, and subjected to heat-mediated antigen retrieval in citrate buffer. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide, and non-specific binding sites were blocked with normal goat serum. Sections were then incubated overnight at 4°C with a primary antibody against CD45R (diluted 1:100). After washing, sections were incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody. Diaminobenzidine (DAB) was used as the chromogen for color development, and hematoxylin was applied for nuclear counterstaining. Stained sections were observed and imaged under a light microscope for analysis.

Immunofluorescence (IF) Staining

For immunofluorescence analysis, paraffin-embedded liver sections were subjected to antigen retrieval and blocked with 5% bovine serum albumin (BSA) to prevent non-specific binding. The sections were then incubated overnight at 4°C with primary antibodies targeting CD24 and CD38 (1:200 dilution). After thorough washing, the sections were incubated with appropriate fluorophore-conjugated secondary antibodies (1:100 dilution) for 1 hour at 37°C in the dark. Cell nuclei were counterstained with Hoechst 33258. Finally, the slides were mounted with an anti-fade mounting medium and visualized using a confocal laser scanning microscope (Olympus SpinSR10).

Flow Cytometry

Liver mononuclear cells (LMNCs) were isolated by ex vivo collagenase perfusion, followed by mechanical dissociation, filtration through a 70- μ m cell strainer, and density gradient centrifugation in 33% Percoll. After red blood cell lysis, cell viability was determined by trypan blue exclusion. For immunophenotyping, single-cell suspensions (1×10^6 cells) were stained with a viability dye (PerCP/Cyanine7-AAD) and fluorophore-conjugated antibodies against mouse CD45 (APC) and CD19 (PE) for 30 min at 4°C in the dark. Cells were then washed, resuspended in staining buffer, and analyzed immediately using a flow cytometer. Data were processed with FlowJo v10.8.

Statistical Analysis

Data are expressed as the mean $\pm$ standard deviation (SD). Statistical comparisons were performed using GraphPad Prism software (version 10.1.2). Differences among multiple groups were analyzed by one-way analysis of variance (ANOVA), followed by the Least Significant Difference (LSD) post hoc test for pairwise comparisons. A p-value of less than 0.05 was considered statistically significant. All experiments were independently repeated at least three times.

Results

Hypoxia Preconditioning Enhances the Expression of VEGF, c-MET, and HIF-1 α in BMSCs

To assess the effect of hypoxia preconditioning (HP) on BMSC function, we first analyzed the expression of key genes and proteins. Western blot analysis revealed that HP (1% O₂ for 24 h) significantly increased the protein level of HIF-1 α compared to normoxic culture (21% O₂; $P < 0.001$), confirming activation of the cellular hypoxic response (Fig. 1A, B). At the mRNA level, RT-qPCR demonstrated that HP markedly upregulated the expression of VEGF, CXCR4, and SDF-1 ($P < 0.001$) while downregulating CCL2 ($P < 0.001$; Fig. 1C), indicating an altered chemokine profile alongside enhanced angiogenic signaling. Western blot further validated that protein levels of VEGF, c-MET, HIF-1 α , and the proliferation marker PCNA were all significantly higher in HP-treated BMSCs than in controls ($P < 0.001$; Fig. 1D). These results indicate that HP systematically upregulates multiple tissue-repair-related molecules in BMSCs through HIF-1 α pathway activation, establishing a molecular foundation for their subsequent therapeutic application.

Hypoxia-Preconditioned BMSCs Ameliorate Liver Injury in Acute Liver Failure

To evaluate the therapeutic effect of HP-BMSCs in vivo, we administered BMSCs via tail vein injection to mice with D-GalN/LPS-induced acute liver failure (ALF; Fig. 2A). Compared to the ALF model group, mice receiving HP-BMSCs showed significantly lower serum levels of ALT, AST, and TBIL ($P < 0.05$; Fig. 2B),

indicating improved liver function. Gross examination revealed marked hepatic congestion and swelling in ALF mice, which were visibly alleviated after BMSC infusion, with the most notable improvement observed in the HP-BMSC group (Fig. 2C). Histopathological analysis by H&E staining further confirmed extensive hepatocyte necrosis and inflammatory infiltration in ALF mice. BMSC transplantation attenuated these injuries, and the protective effect was more pronounced in the HP-BMSC group than in the normoxia-BMSC group (Fig. 2D). Taken together, these results demonstrate that HP-BMSCs exhibit superior efficacy in mitigating liver injury and promoting tissue repair in ALF mice.

Hypoxia-Preconditioned BMSCs Promote Liver Regeneration in Acute Liver Failure

We next investigated whether HP-BMSCs influence the hepatic regenerative microenvironment. Western blot analysis showed that HP-BMSC infusion significantly increased the protein levels of VEGF, c-Met, and HGF- α in the livers of ALF mice compared to normoxic BMSC infusion ($P < 0.01$; Fig. 3A), indicating enhanced activation of regeneration-associated pathways. RT-qPCR analysis of liver tissue revealed that HP-BMSC treatment upregulated the expression of pro-regenerative factors (VEGF, PDGF, CXCR4) while downregulating pro-inflammatory cytokines (IL-6, TNF- α) and upregulating the anti-inflammatory cytokine IL-10 ($P < 0.001$; Fig. 3B). Consistent with these findings, serum ELISA confirmed a more pronounced decrease in IL-6 and increase in IL-10 in the HP-BMSC group ($P < 0.01$; Fig. 3C). Collectively, these results demonstrate that HP-BMSCs not only mitigate injury but also actively reshape the hepatic milieu toward a pro-regenerative and anti-inflammatory state, thereby facilitating tissue repair.

Hypoxia-Preconditioned BMSCs Reduce B Lymphocyte Infiltration in Liver Tissue

To explore the immunomodulatory role of HP-BMSCs, we analyzed B lymphocyte infiltration in liver tissue. Immunohistochemistry and flow cytometry revealed that BMSC infusion reduced the number of infiltrating B lymphocytes compared to the ALF model group, with a more significant reduction in the HP-BMSC group than in the normoxia-BMSC group ($P < 0.05$; Fig. 4A, B). In parallel, immunofluorescence staining showed a marked increase in CD24 + CD38+B cells in the HP-BMSC group (Fig. 4C), suggesting a shift in B-cell subset composition. These results indicate that HP-BMSCs can modulate intrahepatic immune infiltration, notably by altering the distribution of B lymphocyte subsets, which may contribute to attenuating immune-mediated injury and supporting a reparative microenvironment in ALF.

Discussion

Acute liver failure (ALF) represents a critical medical condition with high mortality and limited therapeutic options beyond liver transplantation. Mesenchymal stem cell (MSC) therapy, particularly using bone marrow-derived MSCs (BMSCs), holds significant promise due to its paracrine, immunomodulatory, and regenerative capacities. A central challenge, however, lies in enhancing BMSC

survival and function within the hostile microenvironment of the failing liver. Preconditioning strategies, such as hypoxia, have emerged as a key approach to prime MSCs before transplantation. Consistent with studies on other MSC types, such as human amniotic MSCs where hypoxia preconditioning was shown to enhance proliferation, migration, and homing via the HGF/c-Met axis [13], our study demonstrates that hypoxia preconditioning (HP) is a potent strategy to prime BMSCs, significantly boosting their therapeutic efficacy in a murine ALF model. Further supporting the broad applicability of hypoxic priming, research on hypoxic mesenchymal stem cell-derived exosomes has revealed that hypoxia preconditioning enriches exosomal miR-126 via HIF-1 α activation, promoting angiogenesis and fracture healing through the SPRED1/Ras/Erk pathway[14]. This underscores the role of hypoxia in enhancing the paracrine potential of MSCs through exosome-mediated transfer of functional miRNAs. Similarly, in the context of intracerebral hemorrhage, hypoxic preconditioning was found to rejuvenate olfactory mucosa MSCs by upregulating miR-326, which attenuates cellular senescence and enhances autophagy via the PTBP1/PI3K axis, thereby improving cell survival and neuroprotection[15]. Moreover, extracellular vesicles derived from hypoxia-preconditioned olfactory mucosa MSCs have been shown to be enriched with miR-612, which promotes angiogenesis in endothelial cells by targeting TP53 and subsequently activating the HIF-1 α -VEGF signaling axis[16]. These studies collectively highlight that hypoxia preconditioning not only improves MSC viability and function but also robustly modulates the miRNA cargo of their secreted vesicles, activating distinct pro-regenerative and pro-angiogenic pathways critical for therapeutic efficacy across diverse injury models.

The immunomodulatory role of MSCs, particularly regarding B lymphocytes, is an area of growing interest. Recent work by Feng et al. [18] demonstrated in a model of chronic carbon tetrachloride-induced liver fibrosis that MSCs alleviate disease by suppressing the pathogenic functions of intrahepatic B cells, notably their activation, proliferation, and pro-inflammatory cytokine production via exosome-mediated modulation of MAPK and NF- κ B pathways. While that study elegantly establishes B cells as a key cellular target for MSC therapy in a chronic fibrotic setting, our work extends this paradigm into the acute injury context and introduces a crucial preconditioning optimization. We focused on bone marrow-derived cells subjected to hypoxia preconditioning and elucidated a distinct mechanistic axis and a novel immunomodulatory outcome related to B cell subset redistribution. Building on the premise that HP primes BMSCs, we elucidated the underlying molecular circuitry. Cellular adaptation to hypoxia is governed by the master transcriptional regulator hypoxia-inducible factor-1 α (HIF-1 α) [19, 20]. Our data confirm that HP stabilizes HIF-1 α in BMSCs, triggering a pro-regenerative program. A key downstream effect is the robust upregulation of the VEGF/c-MET axis. VEGF, a canonical HIF-1 α target [21, 22], is crucial for promoting angiogenesis and hepatocyte survival. The concurrent upregulation of c-MET suggests a synergistic mechanism. While previous research on amniotic MSCs highlighted the HGF/c-Met pathway in migration [13], and Feng et al. focused on downstream immunomodulatory effectors (exosomes) [18], our findings in HP-BMSCs underscore the prominence of the VEGF/c-MET axis as an upstream molecular switch induced by preconditioning. Consistent with this primed molecular profile, HP-BMSCs conferred superior hepatoprotection in vivo, more effectively reducing serum injury markers and histopathological damage. This functional recovery was underpinned by a reconstituted hepatic

microenvironment: HP-BMSC treatment elevated intrahepatic levels of pro-regenerative factors (VEGF, c-MET, HGF- α) and initiated a profound immunomodulatory shift, significantly reducing the key pro-inflammatory mediator IL-6 [23, 24] and elevating anti-inflammatory IL-10 [25, 26]. This early modulation of the inflammatory landscape is pivotal, as IL-6 is a known potent activator and differentiation factor for B lymphocytes [27–29]. We therefore hypothesized that the cytokine changes induced by HP-BMSCs would directly impact the pathogenic B cell responses in ALF.

Building directly on our observation of an IL-10-enriched, anti-inflammatory milieu, we sought to identify its cellular source and mechanism. An intriguing and novel finding of this study is the specific modulation of intrahepatic B lymphocytes by HP-BMSCs, which distinguishes it from prior work. While Feng et al. reported a broad suppression of intrahepatic B cell infiltration and pro-inflammatory function in a model of chronic fibrosis [18], our study in ALF revealed a more nuanced immunomodulation. We observed a significant reduction in total CD45R⁺ B cell infiltration, aligning with the general immunosuppressive effect of MSCs [30–32]. Critically, however, HP-BMSC treatment induced a concurrent and specific expansion of the intrahepatic CD24⁺ CD38⁺ B cell subset. This phenotype is widely associated with regulatory functions and is a potent producer of the anti-inflammatory cytokine IL-10 [33–35]. This finding suggests that HP-BMSCs not only quantitatively suppress overall B cell infiltration but also actively remodel the B cell compartment, skewing it towards a regulatory, tissue-protective (Breg) phenotype. This qualitative shift provides a plausible cellular mechanism for the elevated IL-10 levels and the enhanced functional recovery we observed. It is particularly significant in the context of acute, fulminant injury like ALF, where a rapid transition from inflammation to resolution is critical for survival. Our data propose a mechanism that extends beyond the mere inhibition of pathogenic immune cells to include active immune reprogramming, potentially offering a more refined and durable therapeutic effect. This adds a new dimension to understanding MSC-mediated immunomodulation in ALF, moving the paradigm beyond well-studied homing, survival, and anti-apoptotic effects to include precise, subset-level regulation of adaptive immunity.

In summary, the superior therapeutic efficacy of HP-BMSCs can be attributed to a multi-faceted repair program, which is synergistically activated by hypoxia preconditioning. This program encompasses enhanced paracrine signaling via the VEGF/c-MET axis to promote angiogenesis and hepatocyte regeneration, a systemic rebalancing of inflammatory cytokines, and—as our novel finding highlights—the active reprogramming of intrahepatic B cell immunity towards a regulatory phenotype. Thus, HP does not merely augment individual BMSC functions but equips them with a coordinated therapeutic toolkit, enabling them to better withstand the hostile ALF microenvironment and orchestrate a more effective repair process.

Several limitations of this study should be considered, which also point to fruitful directions for future research. First, while we focused on the pivotal VEGF/c-MET axis, HP undoubtedly regulates a broader network of genes [33]; comprehensive transcriptomic and proteomic analyses could map this landscape more fully. Second, the direct in vivo fate and spatial distribution of the HP-BMSC secretome were not tracked. Advanced in vivo imaging techniques (e.g., bioluminescence, labeled exosomes) could visualize

these dynamics. Third, and most specific to our key immunologic finding, the precise mechanism by which HP-BMSCs induce the CD24 + CD38+Breg phenotype remains to be defined. It could involve direct cell contact, specific soluble factors, or exosomal signals as demonstrated in other contexts by Feng et al. [18]. Identifying this mediator is a crucial next step. Finally, the translational potential of this strategy requires validation in large animal models of ALF and careful optimization of HP parameters (e.g., duration, oxygen tension) for clinical-grade manufacturing.

Conclusions

Hypoxia preconditioning (HP) significantly enhances the therapeutic efficacy of bone marrow-derived mesenchymal stem cells (BMSCs) in acute liver failure (ALF). HP activates BMSCs through HIF-1 α -dependent upregulation of the VEGF/c-MET axis, enhances paracrine signaling to promote hepatoprotection and regeneration (Fig. 5). Crucially, our findings reveal a novel immunomodulatory mechanism whereby HP-BMSCs actively reprogram intrahepatic B cell immunity, characterized by the specific expansion of regulatory CD24 + CD38+ B cells and a shift toward an IL-10-enriched, anti-inflammatory milieu. This multi-faceted repair program—integrating enhanced paracrine function, cytokine rebalancing, and precise immune subset modulation—enables HP-BMSCs to effectively orchestrate resolution of acute hepatic injury beyond mere suppression of pathogenic responses. Our study suggest hypoxia preconditioning as a robust strategy to optimize stem cell-based therapy for ALF and highlight B cell subset redirection as a promising therapeutic target for modulating adaptive immunity in acute liver diseases.

Statements & Declarations

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Conflict of interest

All authors in this study have no conflicts of interest.

Author contributions

All authors contributed to the study conception and design. M-M L., R-Z S. and Z-Y L. performed research, W-J Q., Y-J G. and W-Q H. analyzed the data. The first draft of the manuscript was written by M-M L. and W-Q H. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

All animal experimental procedures were approved by the Ethics Committee of the The First School of Clinical Medicine, Lanzhou University (approval number: LDYYLL2023-439). Ethics approval is provided in the Supplementary Materials. All procedures were conducted following relevant guidelines and regulations.

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Data availability

The authors declare that all data supporting the findings of this study are available in the article.

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Table 1

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Figures

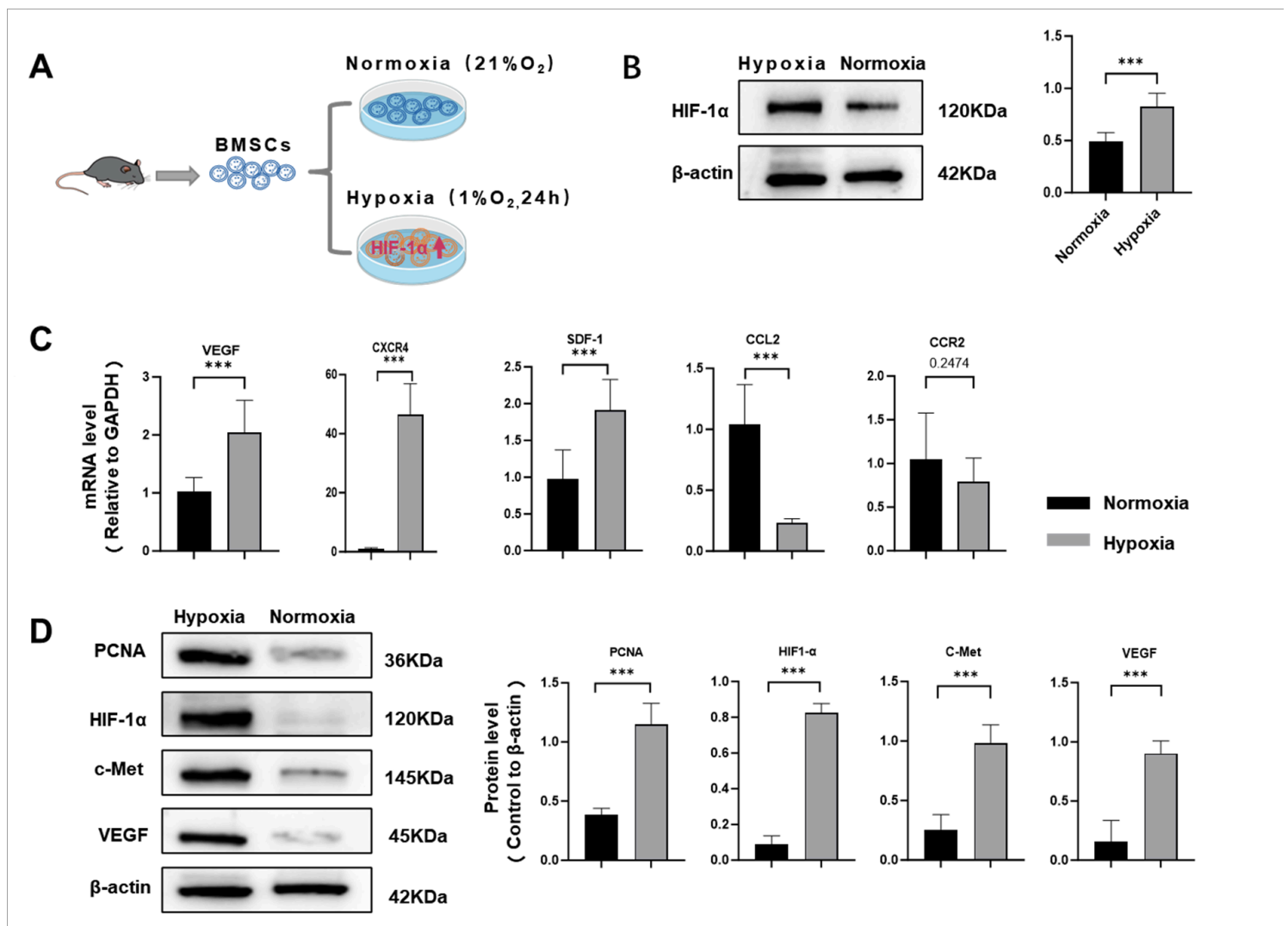


Figure 1

Hypoxia preconditioning enhances the expressions of VEGF, c-Met and HIF-1α in bone marrow mesenchymal stem cells (BMSCs). (A) Schematic diagram of hypoxia preconditioning in mouse BMSCs; (B) Western blot analysis of HIF-α in mouse BMSCs after hypoxia preconditioning; (C) RT-qPCR assays demonstrated that hypoxia preconditioning upregulated the mRNA expressions of VEGF, CXCR4 and SDF-1, while downregulated those of CCL2 and CCR2 in BMSCs; (D) Western blot analysis revealed the upregulated expressions of PCNA, c-Met and VEGF in BMSCs following hypoxia preconditioning. Mean±SD. * $P < 0.05$, *** $P < 0.001$.

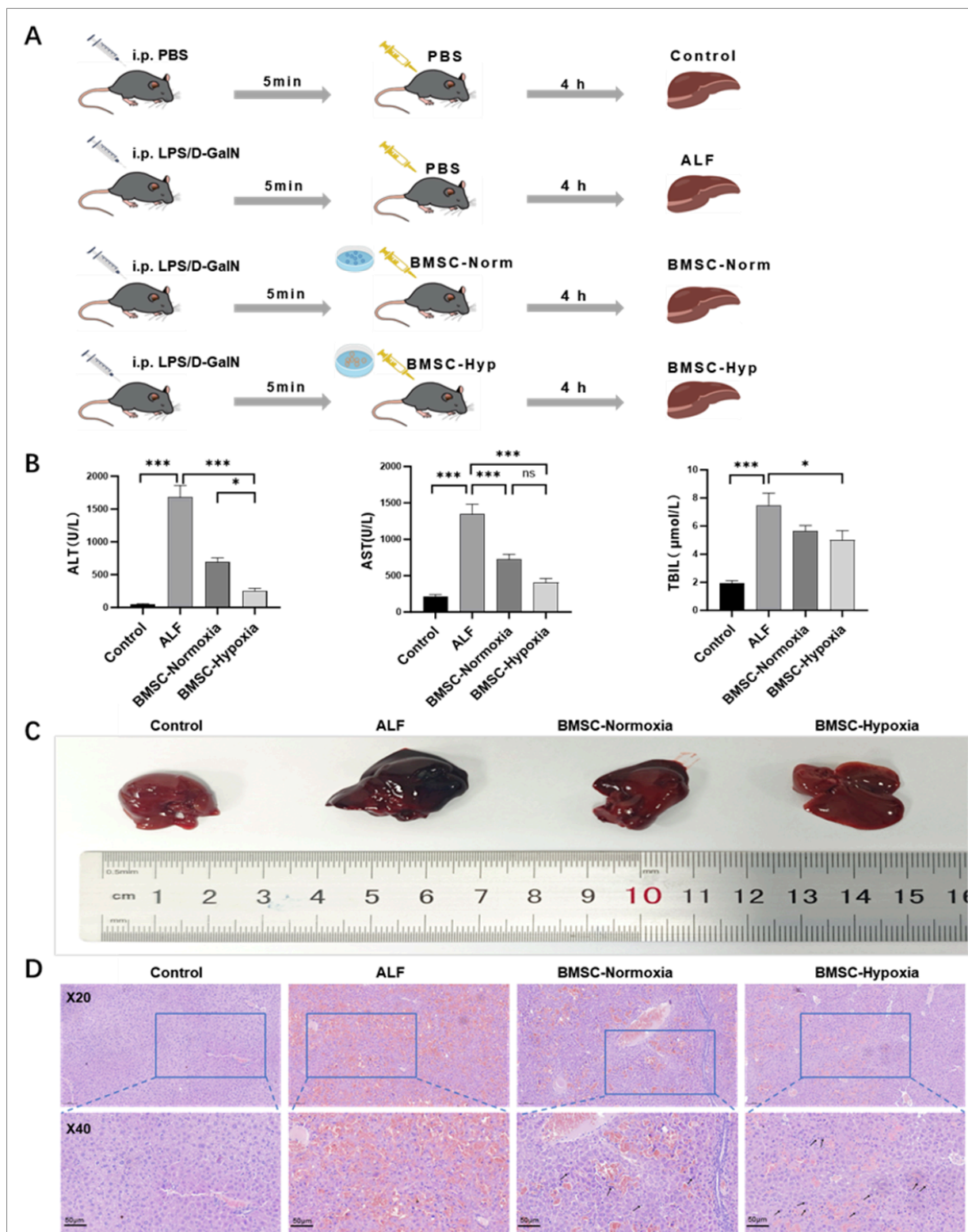


Figure 2

Hypoxia-preconditioned BMSCs ameliorate acute liver failure-induced liver injury. (A) Schematic diagram of animal grouping and experimental procedures. (B) Compared with the acute liver failure (ALF) model group, mice treated with hypoxia-preconditioned BMSCs showed a significant decrease in liver function markers ALT, AST, and TBIL. (C) Gross examination of livers revealed marked congestion and enlargement in the ALF model group compared with the normal group. Transplantation of BMSCs

alleviated hepatic congestion and reduced liver size, with a more pronounced improvement in the hypoxia-preconditioned BMSCs group. (D) HE staining demonstrated extensive hepatic degeneration, hemorrhagic necrosis, and inflammatory cell infiltration in ALF mice. BMSCs administration markedly attenuated these pathological changes, and hypoxia-preconditioned BMSCs provided more significant protection compared with normoxic BMSCs. Mean $\pm$ SD. n=6. * P <0.05, *** P <0.001.

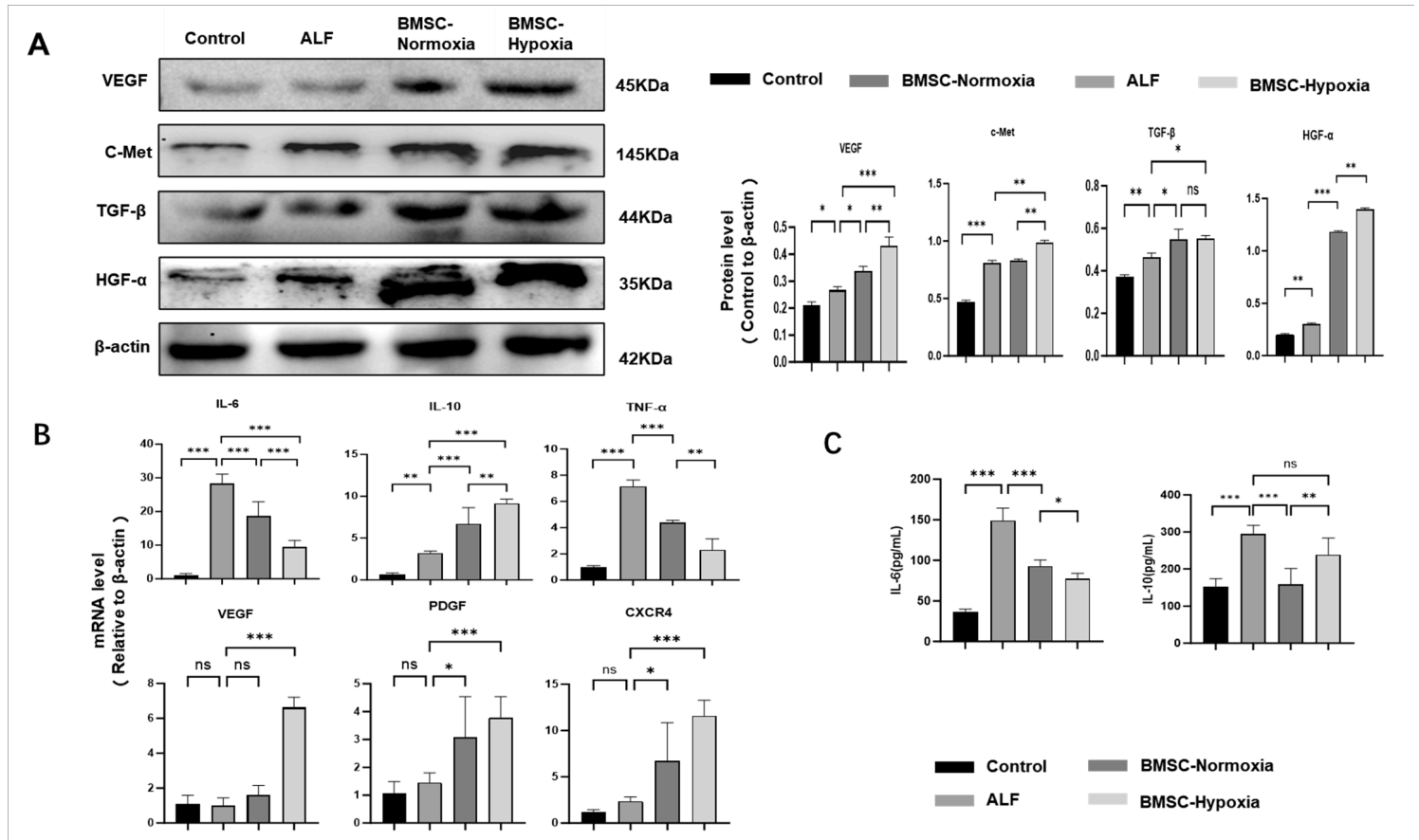


Figure 3

Hypoxia-preconditioned BMSCs promote liver regeneration in acute liver failure. (A) Western blot analysis showed that transplantation of hypoxia-preconditioned BMSCs significantly increased the protein levels of VEGF, c-Met, and HGF-α in liver tissues of ALF mice. (B) RT-qPCR analysis revealed that, compared with normoxic BMSCs, hypoxia-preconditioned BMSCs markedly up-regulated the expression of VEGF, PDGF, and CXCR4, down-regulated IL-6 and TNF-α, and up-regulated IL-10 in liver tissues of ALF mice. (C) ELISA results indicated that serum levels of IL-6 were decreased while IL-10 levels were elevated in BMSCs-treated ALF mice compared with the model group, with more pronounced changes observed in the hypoxia-preconditioned BMSCs group. Mean $\pm$ SD. n=6. * P <0.05, ** P <0.001, *** P <0.001.

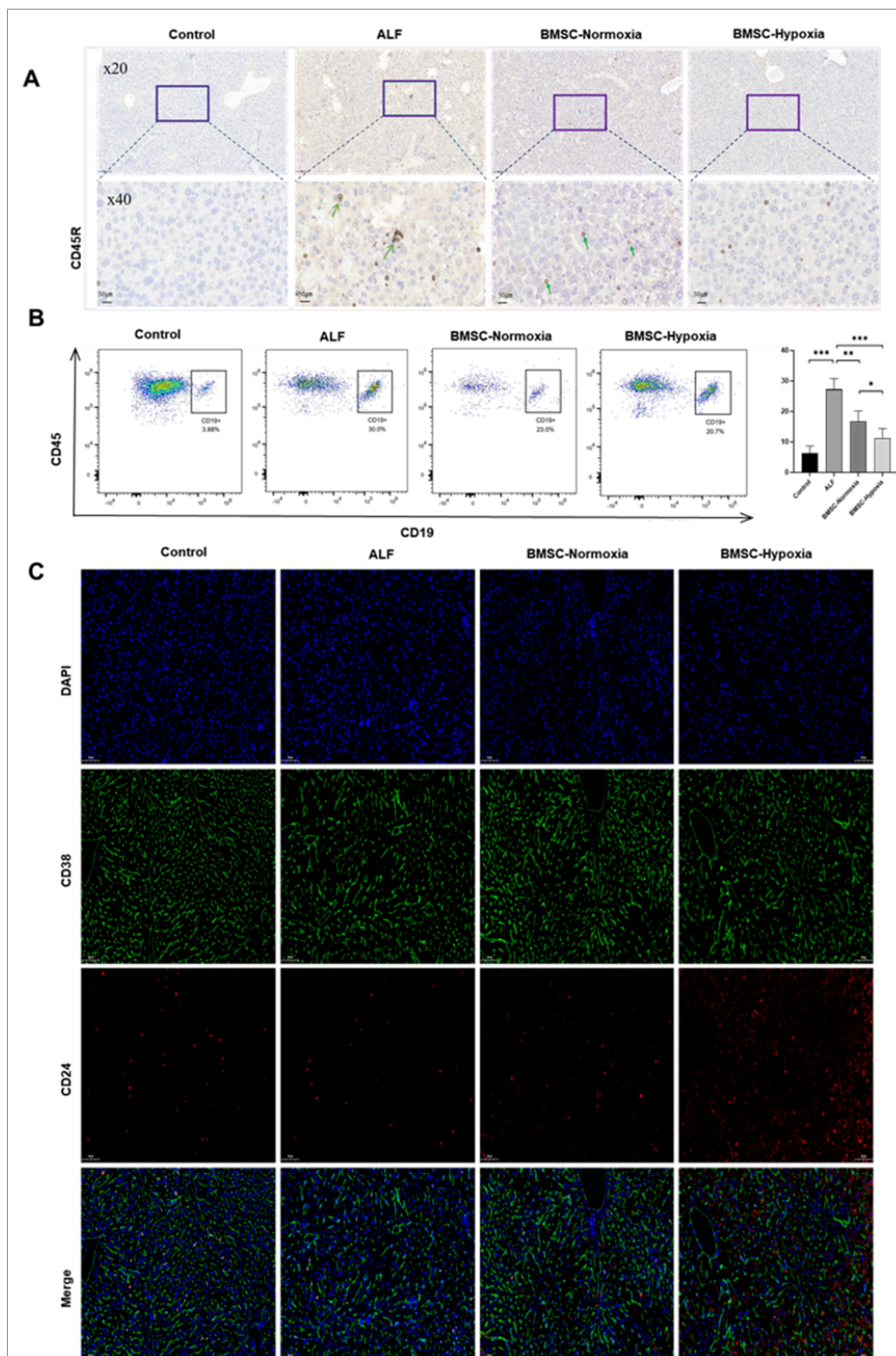


Figure 4

Hypoxia-preconditioned BMSCs reduce B-lymphocyte infiltration in liver tissue. (A)

Immunohistochemical analysis showing changes in B-cell numbers in mouse liver tissue.(B)Flow cytometry quantification of B-lymphocyte infiltration in mouse liver tissue.(C)Immunofluorescence staining indicates a significant increase in CD24+CD38+ B-cell positivity in the hypoxia-preconditioned group.

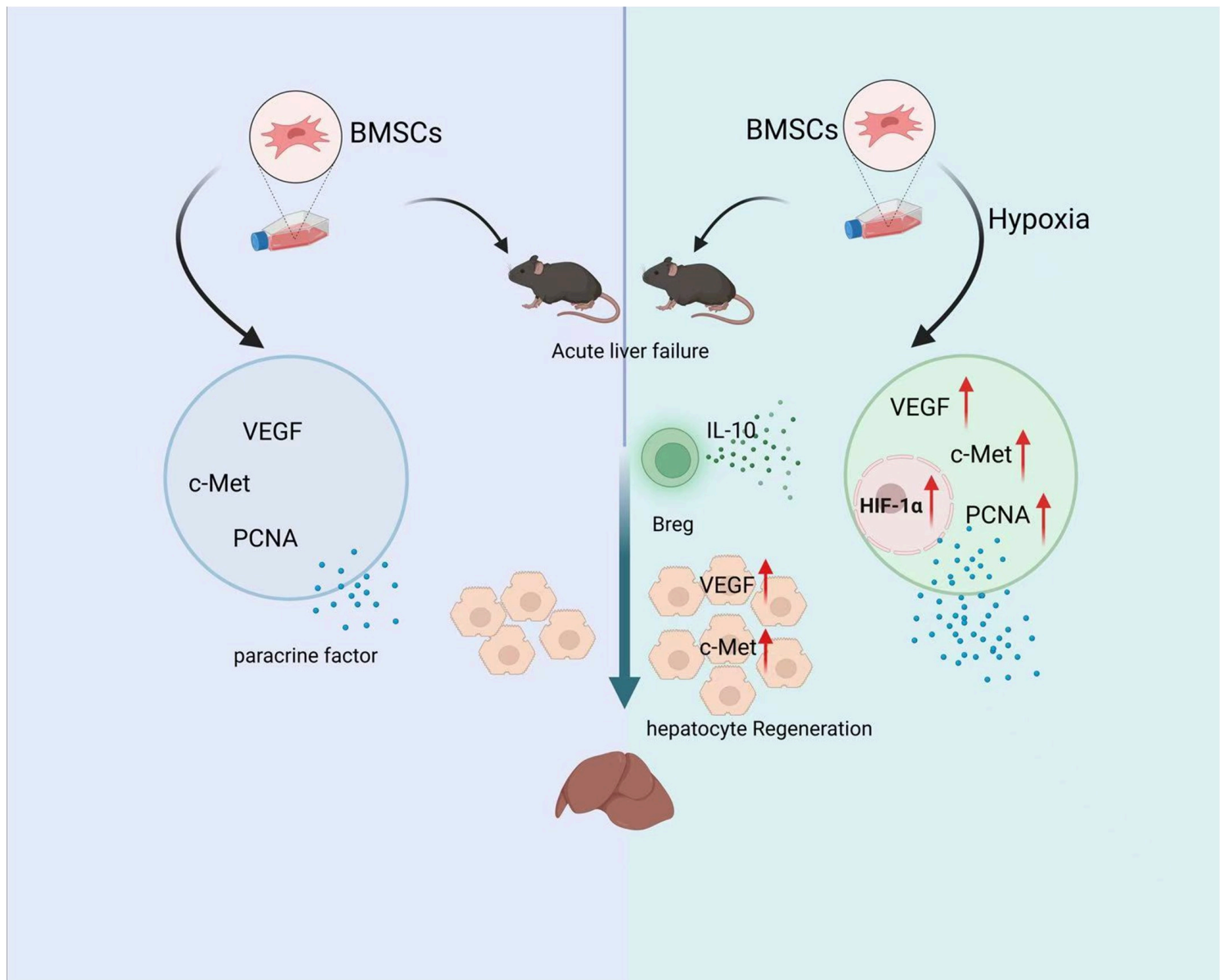


Figure 5

Mechanism of HP-BMSC therapy in ALF. Hypoxia preconditioning (HP) stabilizes HIF-1α in BMSCs, activating the pro-regenerative VEGF/c-MET axis. Transplanted HP-BMSCs exert therapeutic effects through: (1) enhanced paracrine secretion (VEGF, HGF-α) for regeneration; (2) inflammatory rebalancing (↓IL-6, ↑IL-10); and (3) B cell immunomodulation, reducing total infiltration while expanding regulatory CD24+CD38+B cells (Bregs). These coordinated actions mitigate liver injury and promote recovery.

Supplementary Files

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