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Original Research

Histopathological and Immunohistochemical Mechanisms of Bone Marrow-Derived Mesenchymal Stem Cells in Reversion of Gastric Precancerous Lesions

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Abstract

Background: Gastric cancer (GC) stands as one of the most prevalent cancer types worldwide, holding the position of the second leading cause of cancer-related deaths. Gastric lesions represent pathological alterations to the gastric mucosa, with an elevated propensity to advance to gastric cancer. Limited research has explored the potential of stem cells in the treatment of gastric lesions. **Methods**: This study aimed to explore the potential of intravenous transplantation of labeled bone marrow-derived mesenchymal stem cells (BMMSCs) to inhibit the progression of precancerous gastric lesions. **Results**: In the gastric lesion disease model group, the rat tissue exhibited noteworthy mucosal atrophy, intestinal metaplasia, dysplasia, and inflammatory cell infiltration. Following the infusion of BMMSCs, a notable decrease in gastric lesions was found, with atrophic gastritis being the sole remaining lesion, which was confirmed by morphological and histological examinations. BMMSCs that were colonized at gastric lesions could differentiate into epithelial and stromal cells, as determined by the expression of pan-keratin or vimentin. The expression of vascular endothelial growth factor was significantly elevated following BMMSC transplantation. BMMSCs could also upregulate the production of humoral immune response cytokines, including interleukin (IL)-4 and IL-10, and downregulate the production of IL-17 and interferon-gamma, which could be highly associated with the cellular immune response and inflammation severity of the lesions. **Conclusions**: BMMSC transplantation significantly reduced inflammation and reversed gastric lesion progression.

Keywords: mesenchymal stem cells; gastric lesions; intravenous administration; immune response; tumor reversion; animal model

1. Introduction

Precancerous gastric lesions, hereafter referred to as gastric lesions, are identifiable pathological changes to the gastric mucosa that are more likely to develop into gastric cancer (GC) [1]. Intestinal metaplasia and dysplasia are two common types of gastric lesions caused by long-term injury and repair cycles. Gastric carcinogenesis can be described as a series of sequential phases, and it is widely accepted that GC occurs following the mucosal atrophy-intestinal metaplasia-dysplasia disease course. Globally, GC is one of the most common malignancies and is also the fourth leading cause of cancer-related deaths [2].

Emerging strategies in stem cell transplantation hold promise as an effective approach to alleviate patient suffering and enhance the treatment of a diverse range of diseases and injuries. Mesenchymal stem cells (MSCs) represent a particularly promising tool for innovative clinical concepts supporting cellular therapy. Initially identified in bone marrow (BM), MSCs demonstrate robust self-renewal and multilineage differentiation potential [3]. Furthermore, they express various surface markers typical of stromal cells, endothelial cells, and epidermal cells [4]. MSCs exhibit the ability to migrate towards ischemic or injured tissues and organs through the circulatory system [5]. They can also be recruited directly to gastrointestinal tissue, participating in both physiological regeneration and pathophysiological repair [6].

Numerous cancer types arise from chronic inflammation, as it has the potential to alter the local microenvironment, thereby increasing the risk of cancer [7,8]. However, a microenvironment conducive to tissue repair serves as the foundation for disease recovery. Typically, MSCs can regulate immune responses, trigger the production of growth factors and secretion of cytokines to inhibit an inflammatory response [9], improve the microenvironment [10], and control the differentiation of stem cells [11]. However, the biological effects of colonized MSCs may be weakened and their proper differentiation may be compromised when the microenvironment changes during sustained inflammation [12]. MSC transfusion has been exhibited to reverse inflammation-related gastric lesions by regulating and improving the microenvironment [13].

Previous studies have explored predictive factors for prolonged remission, and in young patients with type 1 di-

abetes mellitus, residual beta cell function predicts clinical response after autologous hematopoietic stem cell transplantation [14,15]. A previous clinical study on the treatment of ulcerative colitis (UC) using autologous bone marrow whole stem cell transplantation has exhibited to be a safe and effective alternative treatment [16]. As there is strong evidence that bone marrow-derived mesenchymal stem cells (BMMSCs) contribute to the repair of various gastrointestinal injuries [17], the present study aimed to examine their ability to repair gastric lesions. By injecting BMMSCs into the tail vein of rats with a gastric lesion model, the colonization and differentiation of BMMSCs at sites of local gastric lesions were confirmed. The effects of BMMSCs on inflammation-associated cytokines were also investigated, and BMMSCs could serve as optimal candidates for stem cell therapy aimed at impeding the advancement of gastric lesions, leveraging anti-inflammatory and immunomodulatory mechanisms. This study may provide preliminary data and establish the foundation for BMMSC-based therapy in the context of gastric precancerous lesions.

2. Materials and Methods

2.1 Isolation of MSCs from Rat BM

BM aspirates were obtained from the tibia and femur of male Wistar rats using 21-gauge needles containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 1% antibiotics and antimycotics. After filtering through a nylon mesh (50 µm) and washing with DMEM, the cells were centrifuged, re-suspended, and plated in a petri dish (100 mm) in DMEM containing 1% heat-inactivated fetal calf serum. Cells were incubated at 37 °C with 5% CO_2 in a humidified chamber. After 72 h, the adherent cells were gently washed using phosphate-buffered saline (PBS) before adding fresh culture medium, and the medium was replaced every three days. At approximately 80–90% confluency, the cells were detached using trypsin (0.25%) containing Ethylene Diamine Tetraacetic Acid (EDTA) (1 mmol/L). The cells were subsequently titrated and passaged. The cells were used for experiments after the P3 generation, when the morphology of the BMMSCs remained consistent.

2.2 Labeling of MSCs by CM-dil

The appropriate concentration of CellTracker™ CM-Dil dye (1 µg/mL; C7000; Invitrogen Corp., Carlsbad, CA, USA) was established as suitable through preliminary experiments. Cells from the third passage were harvested and counted, and CM-Dil (3 µL) working solution was utilized to stain 1×10^6 cells following the manufacturer's instructions. The cells were incubated at 37 °C for 5 min, and were then incubated at 4 °C for an additional 15 min. After labeling, the cells were washed with PBS and resuspended in fresh medium.

2.3 Animal Model of Gastric Lesions and Cell Transplantation

Wistar rats of the male gender, aged 6 weeks (160–180 g) were provided by the Laboratory Animal Center of the Fifth Medical Center, Chinese PLA General Hospital (Certification number SCXK-JUN 2007-004). The room temperature was set at 20 ± 2 °C, and the humidity was 60–70%. A 12-h day–night cycle was maintained, and the rats had free access to standard diet and water. All animals were acclimated for 3 days prior to the experiments. All experimental procedures were conducted with the approval of the Ethics Committee of the Animal Facility of the Chinese PLA General Hospital and adhered to the established guidelines for the care of laboratory animals.

Six-week-old male Wistar rats were randomly divided into two groups: control group (n = 4) and model group (n = 22). Rats in the control group were fed standard rat chow and tap water *ad libitum*. In addition to standard rat chow, rats in the model group also had access to a fresh solution of MMNG (N-methyl-N′-nitro-N-nitrosoguanidine; 100 mg/L; MO527, TCI, Tokyo, Japan) *ad libitum*. The systemic influence of MNNG was initially explored by assessing appetite, body weight, and activity of each rat. When the model was established, three rats were anesthetized and killed every month for macroscopic and microscopic analyses of the MNNG-induced gastric lesions. Ten months after the initiation of the model, increased inflammatory cell infiltration, glandular epithelial atrophy, reduction of intrinsic glands, structural disorder of some glands, and irregular shapes were observed in the gastric mucosa of rats in the model group. Animals in the gastric lesion model group were subsequently randomly divided into two additional groups: the transplantation group (n = 11) and the nontransplantation group (n = 11). Rats in the transplantation group received transplantation of CM-dil labeled MSCs (1 mL) at a concentration of 3×10^6 cells/mL via tail vein injection once per week for three consecutive weeks. Rats in the nontransplantation group received an equal volume of physiological saline through the tail vein.

2.4 Blood and Gastric Sample Processing

Rats in the control and nontransplantation groups were killed one week after the last stem cell transplantation, and blood was collected from the heart chambers after intraperitoneal anesthesia with 10% chloral hydrate (0.3 mL/100 g). After the blood was centrifuged for 10 min at 3000 rpm, serum was collected and stored at –20 °C. In addition to blood, the entire glandular stomach was resected and incised rapidly along the greater curvature. For background staining and immunohistochemistry, the gastric tissue was fixed with 4% paraformaldehyde before paraffin-embedding and sequential slices were obtained. Lesions, inflammatory cell infiltration, atrophy, intestinal metaplasia, and dysplasia were assessed based on histological criteria from hematoxylin and eosin-stained gastric mucosa

samples. The pathological changes were graded as mild, moderate, or severe based on a pathological grading system [18]. In the transplantation group, rats were anesthetized and killed for macroscopic and microscopic analyses after the last injection of labeled cells. Samples of the glandular stomach that were left after the previously described tissue collection were embedded in Tissue-Tek OCT compound (4583, Sakura Co., Ltd., Tokyo, Japan) and frozen in liquid nitrogen for subsequent cryostat sectioning and immunofluorescence staining.

2.5 Immunofluorescence

Continuous frozen sections (7 µm) were permeabilized with Triton X-100 (1%) in PBS (pH 7.4) for 20 min, followed by incubation in blocking buffer containing goat serum (10%) for 30 min. For immunofluorescence staining, the sections were subsequently treated with anti-vimentin antibody (SC-6260, monoclonal mouse, 1:200 dilution, Santa Cruz Biotechnology) or pan-keratin antibody (4545S, monoclonal mouse, 1:300 dilution, Cell Signaling Technology, Danvers, MA, USA) overnight at 4 °C. Fluorescein isothiocyanate-conjugated goat anti-mouse IgG antibody (SC-2010, Santa Cruz Biotechnology, Dallas, TX, USA) was utilized as the secondary antibody (1:100; Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and sections were incubated for 1 h at 37 °C. Nuclei were visualized using 4′6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich, St. Louis, MO, USA).

2.6 Immunohistochemistry

Immunohistochemistry was performed to detect the expression level of vascular endothelial growth factor (VEGF). Briefly, paraffin-embedded sections were deparaffinized and rehydrated. After blocking endogenous peroxidase activity with H_2O_2 (0.3%) for 30 min, the sections were subsequently incubated with rabbit anti-VEGF antibody (ab46154, 1:200 dilution; Abcam, Cambridge, UK) overnight at 4 °C. After washing with PBS (pH 7.4), the sections were incubated with anti-rabbit immunoglobulins (ZDR-5118, ZBGB-BIO, Zhongshan, China) for 30 min, and then allowed to react with streptavidin conjugated to horseradish peroxidase for 30 min. Antibody binding was visualized by incubation with 3.3′-diaminobenzidine chromogen (Dako, Carpinteria, CA, USA). Hematoxylin was used for nuclear counterstaining.

2.7 Flow Cytometric Analysis of Cytokines

The presence of interleukin (IL)-17 (BMS8635FF; eBioscience, San Diego, CA, USA), IL-4 (51-9004113; BD Biosciences, Franklin Lakes, NJ, USA), IL-10 (51-9004112, BD Biosciences), and interferon-gamma (IFN-γ; 51-9004111, BD Biosciences) in the serum was detected by flow cytometry following the manufacturers' instructions.

2.8 Pathological Judgment Criteria

Two pathologists specializing in pathology for more than 5 years assessed gastric tissue lesions under light microscopy. The diagnostic criteria for histological changes in the gastric mucosa refer to the classification and diagnostic criteria of experimental gastric precancerous lesions. The pathological indicators were summarized as follows, encompassing the assessment of gastric mucosal injury, inflammation, atrophy, intestinal metaplasia, and atypical hyperplasia.

Gastric mucosal injury: The degree of gastric mucosal injury was categorized into four levels.

Mild: Superficial injury of gastric mucosal surface epithelial cells, scored 1 point; Moderate: Damage above the gastric pit, manifested as superficial erosion, scored 2 points; Severe: Damage below the gastric pit, manifested as deep erosion, scored 3 points; Ultra Severe: Full-thickness necrosis and desquamation of gastric mucosa, manifested as acute ulcer, scored 4 points.

Gastric mucositis was semi-quantitatively assessed. Five visual fields were observed for each gastric body and gastric antrum slice under low magnification. Based on the degree of inflammatory cell infiltration, it was classified into the following levels.

Grade 0: No inflammation, scored 0 points; Grade 1: Multiple chronic inflammatory cell infiltrates in the gastric mucosal epithelium or at the base of the proper gland, scored 1 point; Grade 2: More inflammatory cells infiltrating from the gastric mucosal epithelium to the mucosal muscularis, scored 2 points; Grade 3: Piles of inflammatory cell aggregates in the gastric mucosa, scored 3 points.

2.9 Source of Animals

Wistar rats of the male gender, aged 6 weeks (160–180 g), and meeting the criteria of Specific Pathogen-Free (SPF) status were utilized in the study. These rats were obtained from and maintained by the Animal Laboratory Center of the Fifth Medical Center, Chinese PLA General Hospital, under license number SCXK (Military) 2007-004. All experimental procedures were conducted with the approval of the Ethics Committee of the Animal Facility of the Chinese PLA General Hospital and adhered to the established guidelines for the care of laboratory animals [19].

2.10 The Measurement of the Optical Density and Statistical Analysis

Five different fields of view were randomly selected from each immunohistochemical section under a high-power microscope (400×). According to the observations, the cells with brownish-yellow particles in the cytoplasm were positive for VEGF. Image-pro plus 5.1 software (Media Cybernetics, Bethesda, MD, USA) was used to analyze the optical density and area of immunohistochemical positive signals in the selected visual field, and to calculate the average optical density value of VEGF (average optical

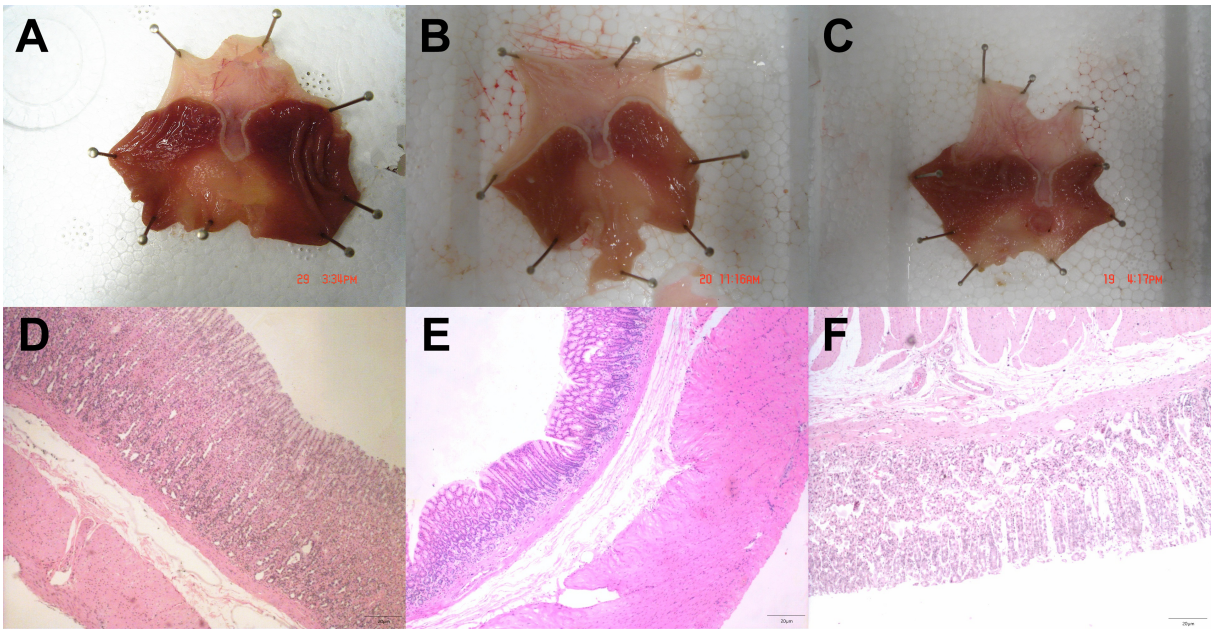

Fig. 1. Gastric precancerous lesions were less severe in mice treated with bone marrow-derived mesenchymal stem cells (BMMSCs) that were administered through the tail vein of rats. (A–C) representative images from morphological observations of the rat gastric mucosa in normal (A), gastric lesion model (B) and BMMSC transplant (C) groups. (D–F) Hematoxylin and eosin-stained images from frozen pathological section of rat gastric mucosa in normal (D), gastric lesion model (E), and BMMSC transplant (F) groups.

density value = cumulative optical density/area). A higher average optical density value indicates a stronger expression.

All the data were expressed as the mean ± standard deviation and analyzed using SPSS 16.0 software (IBM Corp., Armonk, NY, USA). For making comparisons among multiple groups, the repeated measures analysis of variance (ANOVA) test was utilized. A p-value less than 0.05 was considered statistically significant.

3. Results

3.1 MSC Transplantation Reversed Gastric Lesions

In this study, labeled MSCs were injected into the tail vein of rats to assess healing in a model of gastric lesions. Pathological changes of the gastric mucosa were observed in the transplantation group (transplant) and compared to the non-transplantation group (model) and the untreated normal group (control). Both morphological and pathological changes were investigated. The percentages of gastric mucosal pathological changes observed in the three groups of rats are presented in Table 1.

In the model group, atrophic gastritis, intestinal metaplasia, and dysplasia were all observed along with inflammatory cell infiltration. In the transplant group, a significant reduction in gastric lesions was found, with atrophic gastritis being the sole identified lesion. This finding was confirmed through both morphological and histological assessments (Fig. 1).

Table 1. Gastric precancerous lesions were alleviated after treatment with bone marrow-derived mesenchymal stem cells administered through the tail vein of rats (control, n = 4; model and transplant, n = 11).

	Control group	Model group	Transplant group
Normal	50% (2/4)	0% (0/11)	0% (0/11)
Inflammation	50% (2/4)	100% (11/11)	100% (11/11)
Atrophic gastritis	0% (0/4)	64% (7/11)	45% (5/11)
Intestinal metaplasia	0% (0/4)	27% (3/11)	0% (0/11)
Dysplasia	0% (0/4)	73% (8/11)	0% (0/11)

Macroscopically, the normal rat gastric mucosa exhibited good elasticity, a smooth surface, and regular, light-red folds (Fig. 1A). In contrast, the gastric mucosa in rats of the gastric lesion disease model appeared pale with diminished folds (Fig. 1B). The gastric mucosa in the transplant group showed improved morphological changes compared with the model group, and signs of mucosal repair, such as increased mucosal folds were observed (Fig. 1C). Microscopically, the normal rat mucosa exhibited integrated epithelium with regular cell size, well-organized gland cells, and a small number of scattered lymphocytes (Fig. 1D). The gastric lesion model group exhibited clear indications of mucosal atrophy, intestinal metaplasia, and dysplasia (Fig. 1E). Exfoliation of epithelial cells, reduced glands in the lamina propria, and glandular structure damage with noticeable inflammatory cell infiltration were characteristic pathological changes in this group. However, intestinal metaplasia and dysplasia were not found in the trans-

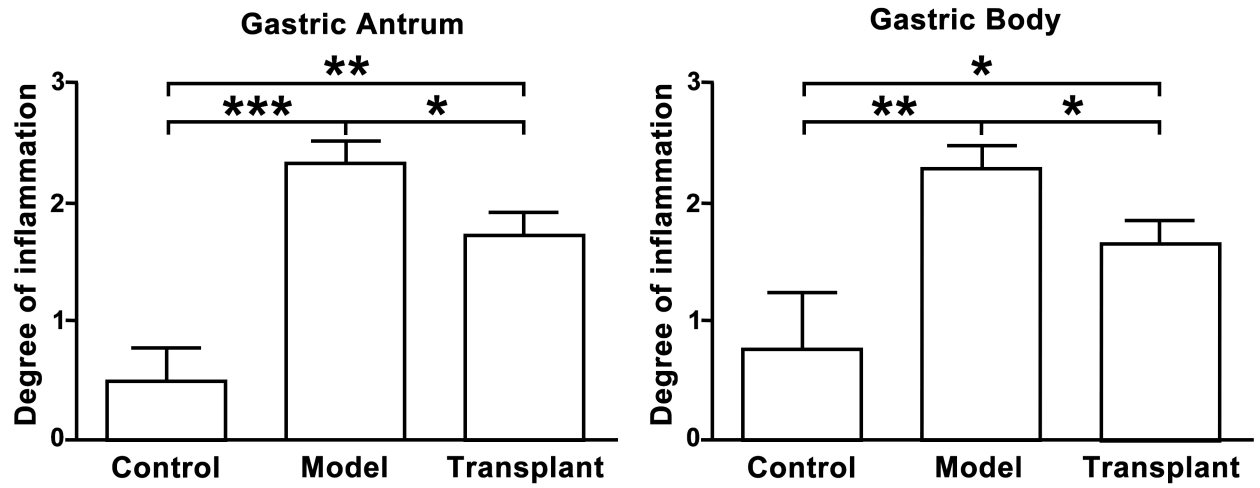

Fig. 2. Inflammation was reduced in the rat stomach during a gastric lesion model after the injection of bone marrow-derived mesenchymal stem cells (BMMSCs). The severity of inflammation within the gastric antrum (left) and gastric body (right) of the control, model, and BMMSC transplant groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

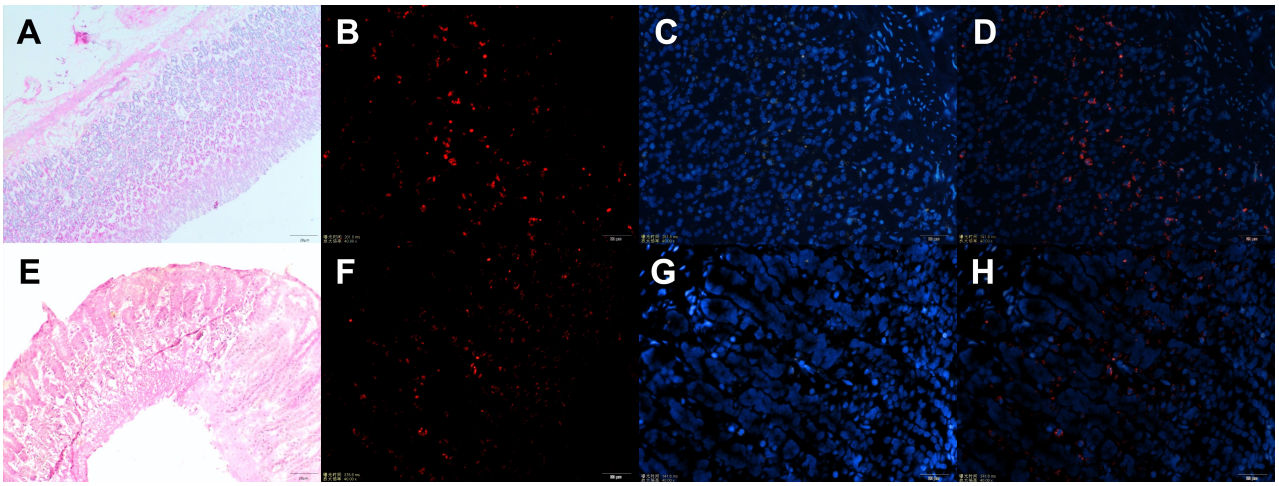

Fig. 3. Injection of bone marrow-derived mesenchymal stem cells (BMMSCs) could be colonized in gastric precancerous tissues. Representative images from frozen pathological sections of rat gastric body (A–D) and gastric antrum (E–H). (A,E) Hematoxylin and eosin (H&E)-stained image. (B,F) CM-Dil-labeled BMMSCs in precancerous tissues. (C,G) DAPI nuclear staining. (D,H) Merged images. Exposure time, ms; magnification rate, 400×.

plant group (Fig. 1F). Further investigation also revealed that MSC transplantation reduced the severity of gastric mucosal inflammation (Fig. 2). An intensified inflammatory response was detected in both the antrum and body of the gastric mucosa (Fig. 2).

3.2 MSCs Played an Important Role in Tissue Repair

Representative images from frozen gastric tissues following MSC transplantation are illustrated in Fig. 3. Cells with red fluorescence (CM-Dil-labeled cells) were MSCs and were observed at both the gastric body and antrum, while no cells with red fluorescence were found at the gastric fundus. Experiments on control rats were performed in parallel and no cells with red fluorescence were identified in any gastric tissues (Fig. 3). These results demonstrated that MSCs could colonize gastric lesions in rats at the gastric body and antrum.

Using frozen pathological section, two antigens were labeled to identify epithelial and stromal cells, pan-keratin (Fig. 4A–D) and vimentin (Fig. 4E–H), respectively. The results revealed that some MSCs that colonized gastric tissues were differentiated into epithelial cells, while some MSCs retained the expression level of vimentin, an MSC marker.

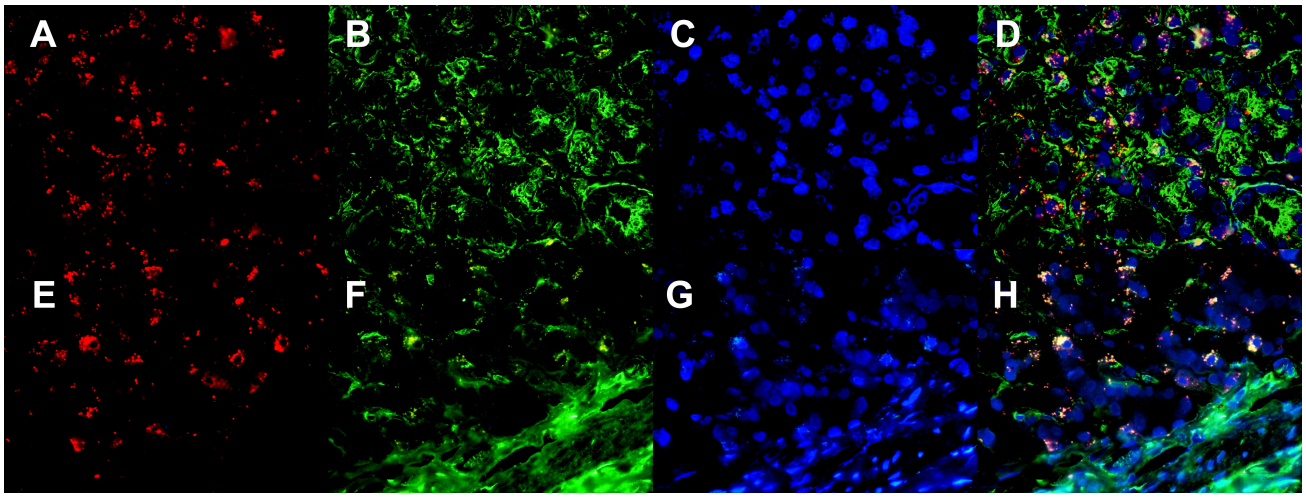

Fig. 4. Bone marrow-derived mesenchymal stem cells (BMMSCs) were colonized in gastric precancerous lesions and differentiated into epithelial cells and mesenchymal cells. Frozen pathological section showing that BMMSCs colonized in gastric precancerous tissues could differentiate into epithelial cells (A–D) and mesenchymal cells (E–H). (A,E) CM-Dil-labeled BMMSCs. (B,F) Pan-keratin (B) and vimentin (F). (C,G) DAPI nuclear staining. (D,H) Merged images.

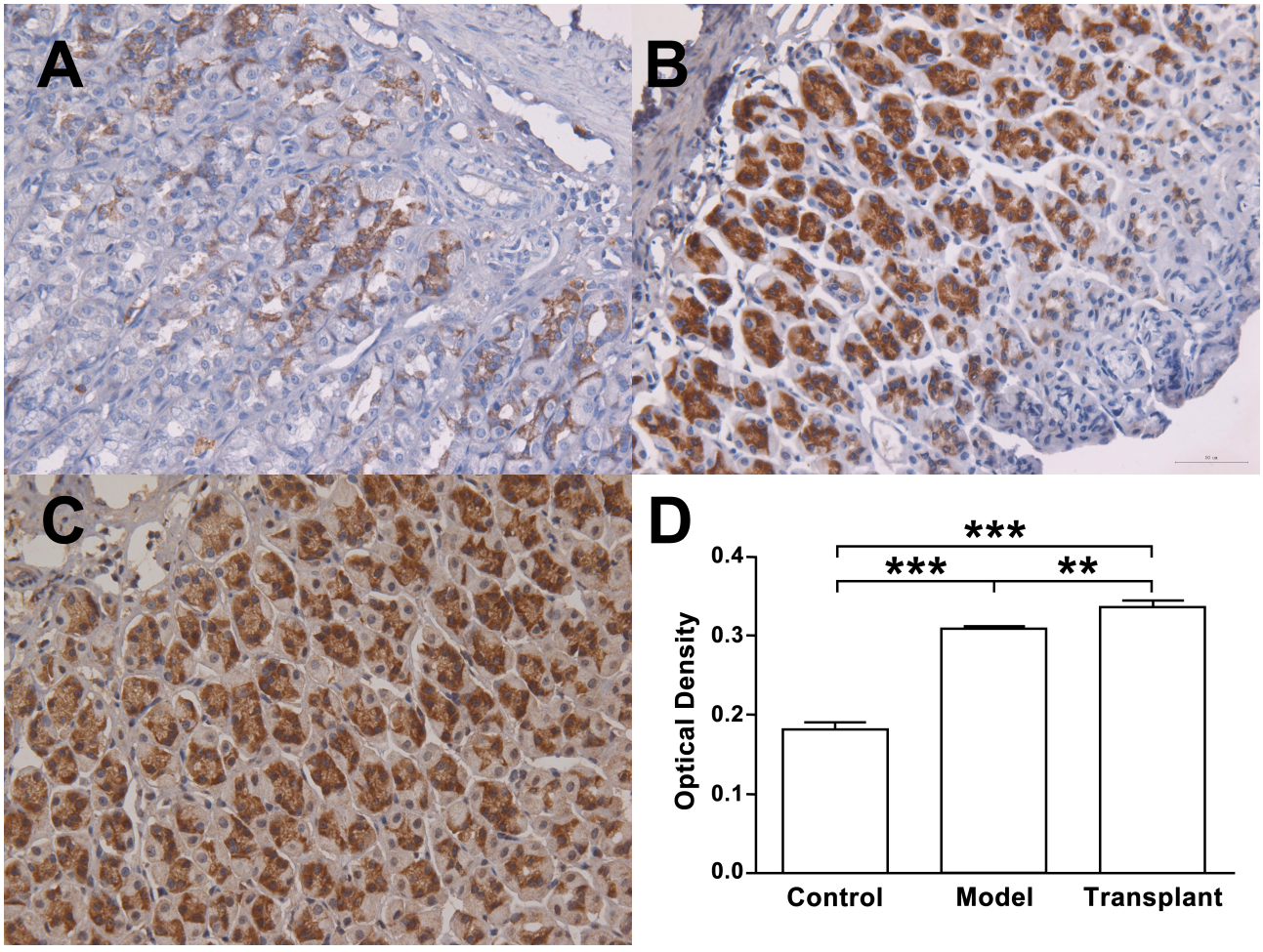

Fig. 5. Bone marrow-derived mesenchymal stem cells (BMMSCs) promoted vascular endothelial growth factor (VEGF) expression in gastric precancerous tissue. (A–C) Immunohistochemical staining of frozen pathological sections from normal, model, and BMMSC transplant groups. (A) Low VEGF expression in control tissue. (B) Enhanced VEGF expression in model tissue. (C) Highest expression of VEGF in transplant group. (D) Optical density was compared among the three groups. n $\geq$ 4. **$p < 0.01$; ***$p < 0.001$.

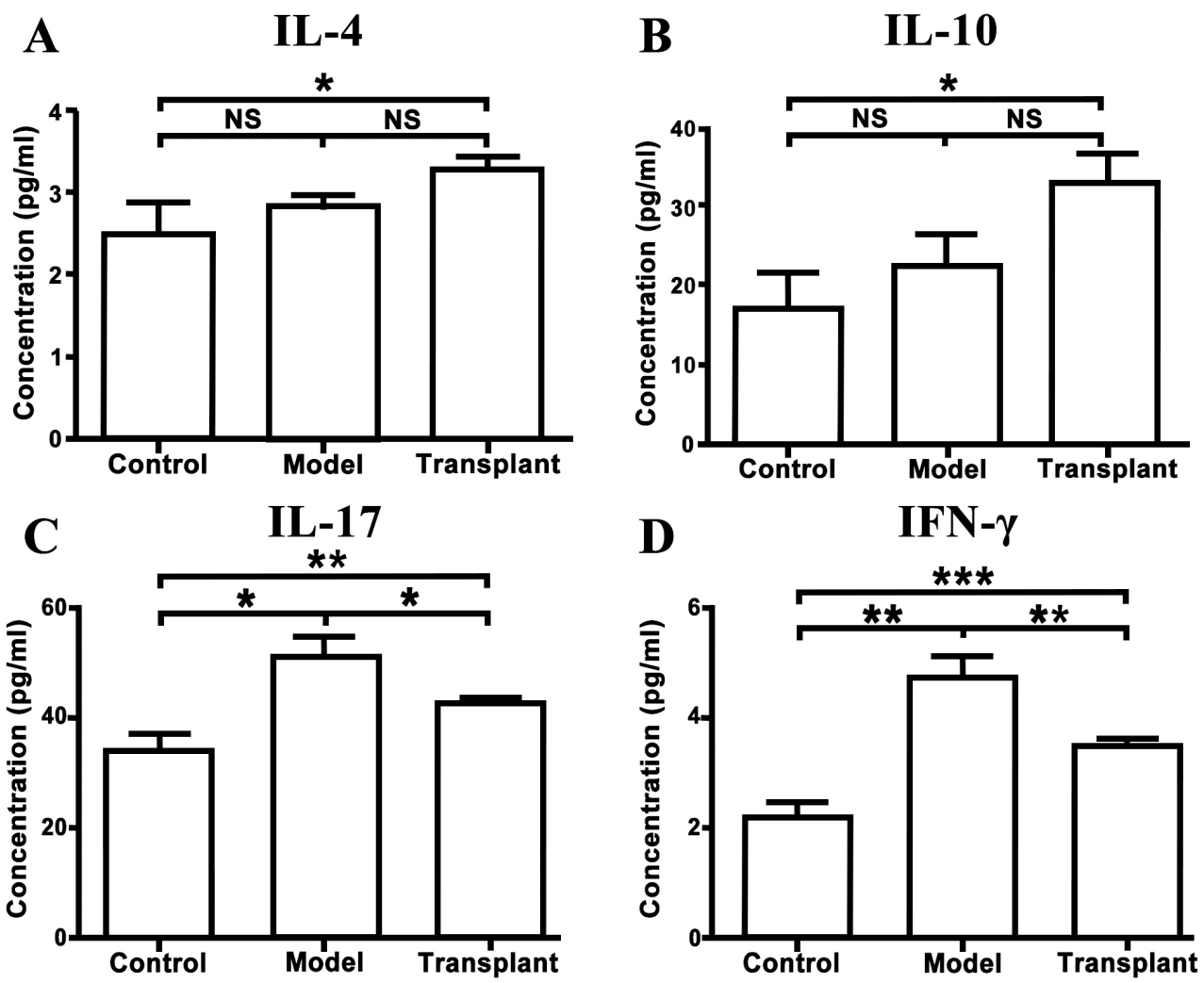

Fig. 6. Bone marrow-derived mesenchymal stem cell (BMMSC) transplantation regulated the serum levels of interleukins and interferon-gamma. Serum levels of IL-4 (A), IL-10 (B), IL-17 (C), and IFN-γ (D) were compared among control, model, and BMMSC transplant groups. n $\geq$ 4. NS: no significance. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

3.3 MSCs Inhibited the Progression of Gastric Lesions through Anti-Inflammatory and Immunomodulatory Mechanisms

The growth of blood vessels can facilitate the repair of lesions. A low expression level of VEGF was identified in normal gastric mucosa (Fig. 5A), and the expression level of VEGF was significantly upregulated in gastric mucosal cells within gastric lesions (Fig. 5B) compared with the normal group ($p < 0.001$). The VEGF expression level in the MSC transplant group was significantly elevated compared with that in both the normal group ($p < 0.001$) and the model group ($p < 0.01$) (Fig. 5C,D). This observation suggested that angiogenesis is evident during the occurrence of gastric lesions, and MSC transplantation further enhances the level of angiogenesis in the injured tissue.

Interleukins and interferons are important cytokines mediating the cellular immune response and are closely associated with carcinogenesis. In the present study, four cytokines potentially related to gastric carcinogenesis were selected and their levels were measured in the serum of rats, including IL-4, IL-10, IL-17, and IFN-γ (Table 2). The results revealed that only serum levels of IL-17 and IFN-γ in the model group significantly increased compared with those in the control group. In contrast, serum levels of IL-4 and IL-10 in the MSC transplant group were significantly higher than those in the control group ($p < 0.05$). Additionally, serum levels of IL-17 and IFN-γ in the MSC transplant group were significantly higher than those in the control group ($p < 0.01$ for IL-17, $p < 0.001$ for IFN-γ), while significantly lower than those in the model group ($p < 0.05$ for IL-17, $p < 0.01$ for IFN-γ) (Fig. 6). IL-4 and IL-10 may reflect the level of the humoral immune response, while IL-17 and IFN-γ are more related to the cellular immune response and inflammation severity of gastric lesions.

Table 2. Serum levels of interleukin (IL)-4, IL-10, IL-17, and interferon-gamma (IFN-γ) in control, precancerous model and bone marrow-derived mesenchymal stem cells (BMMSC) transplant groups. Mean $\pm$ standard error of mean (SEM), n $\geq$ 4.

Group	IL-4	IL-10	IL-17	IFN-γ
Control	2.53 ± 0.36	17.33 ± 4.50	34.30 ± 2.89	2.20 ± 0.28
Model	2.82 ± 0.17	22.73 ± 3.97	51.23 ± 3.81	4.76 ± 0.36
Transplant	3.28 ± 0.17	33.33 ± 3.72	42.84 ± 1.00	3.48 ± 0.11

4. Discussion

In recent years, substantial progress has been achieved in both the research and practical application of MSCs. MSCs have been extensively employed in the investigation of various systemic diseases, such as myocardial infarction, Parkinson's disease, inflammatory bowel disease, autoimmune diseases, diabetes, etc. They have emerged as highly promising seed cells for tissue engineering applications. Notably, multiple studies have demonstrated the *in vitro* differentiation capability of MSCs into gastrointestinal epithelial cells. Furthermore, there is evidence supporting the colonization of MSCs at sites of injured gastrointestinal mucosa, actively participating in the process of tissue repair [20,21]. Local injection of MSCs was also found to facilitate the healing of gastric ulcers [22]. MSCs, when administered via the rat tail vein, are capable of circulating through the bloodstream and reaching various organs and tissues. In these locations, they actively engage in physiological regeneration and contribute to pathophysiological repair [23]. The colonization of MSCs is related to the transplantation method, time, frequency, and tissue microenvironment. When tissue injury is absent, the colonization of MSCs is correlated with the method of transplantation and undergoes redistribution over time. Notably, when administered through veins, MSCs exhibit the highest level of colonization in the lungs, followed by the kidneys, liver, and spleen. In contrast, the lowest level of colonization is found in gastrointestinal tissue [24]. In addition, local injection leads to tissue injury and MSCs are likely to die due to excessive local aggregation [17,25]. The results of the present study revealed that, following MSC injection into the tail vein of rats, MSC colonization was observed in gastric lesions at the gastric body and antrum, while no colonization was found at gastric fundus. This could be attributed to the presence of squamous epithelium, scarce gastric glands, and insufficient blood supply.

Cytokeratin (CK) is one of the characteristic markers of epidermal and epithelial cells [26]. In the present study, it was revealed that MSCs could be differentiated into epithelial cells *in vivo*. Furthermore, it was observed that some MSCs retained the expression level of vimentin (an MSC marker). Migration to and colonization of sites of inflammation and tissue injury are characteristics of MSCs [27]. According to the findings of the present study, it is evident that inflammation plays a role in attracting MSCs to colonize gastric tissues *in vivo*. Subsequently, the colonized MSCs undergo differentiation into the appropriate target cells within the suitable local microenvironment. Therefore, it is noteworthy that the local microenvironment significantly influences the differentiation of MSCs into the required phenotypes following their migration.

BMMSCs display characteristic chemotaxis towards inflammation and sites of tissue damage, displaying the ability to home to sites of chronic inflammation. The local microenvironment can affect the directional differentiation of BMMSCs migrating to local tissues into cells with the correct phenotype, which is a prerequisite for their effectiveness. CK is a characteristic marker of epidermal or epithelial cells, and studies have demonstrated that BMMSCs can differentiate into epithelial cells in both *in vitro* and *in vivo* microenvironments [28,29]. Typically, CK is not expressed by BMMSCs. The detection of CK expression level serves as an indicative marker, suggesting that BMMSCs may have undergone differentiation into epithelial cells. In the current study, pan keratin was used as an indicator of CK. Immunofluorescence assay revealed that staining of BMMSCs coincided with the staining for pan keratin. This indicates that colonized BMMSCs could differentiate into epithelial cells in the *in vivo* microenvironment. Vimentin is a type of intermediate filament protein that is mainly expressed in interstitial cells and nonepidermal cells. Tanaka *et al.* [30] and Hayashi *et al.* [31] demonstrated that in animal models, BMMSCs express vimentin as a mesenchymal cell marker, suggesting their capacity to differentiate into mesenchymal cells. In the present study, vimentin staining was observed in BMMSCs, indicating their ability to differentiate into mesenchymal cells under *in vivo* conditions. This finding aligns with existing literature reports.

Moreover, VEGF is a crucial soluble angiogenic factor with vascular-permeable properties [32]. It plays a vital role in enhancing microvascular permeability to mitigate harmful substances in the stomach, thereby safeguarding the gastric mucosa. VEGF achieves this by regulating the extracellular matrix, stimulating gland secretion, and promoting angiogenesis [33]. MSCs that colonize local tissue secrete VEGF, providing nutritional support to surrounding tissues and playing essential roles in inflammation and injury repair [32]. In the present study, a significant upregulation of VEGF was found in the gastric mucosal tissue in both the model and MSC transplant groups compared with the control group. Notably, the expression of VEGF in the transplant group was significantly higher than that in the model group. These findings suggest that gastric mucosal injury and lesions induced stress and repair responses, stimulating local VEGF expression. This increase in VEGF expression could potentially enhance vascular permeability and gastric mucosal blood flow. MSCs, with the capability of migration to inflammatory lesions, further promoted VEGF pro-

duction within gastric tissues, contributing to tissue repair and defense by improving the vascular microenvironment.

MSCs are stem cells with low immunogenicity, and they play pivotal roles in regulating immunity. Transplanted MSCs can inhibit the activation of T cells [12], reduce local inflammation, and provide a favorable environment for tissue repair [34]. The immunomodulatory activity of MSC is closely associated with IFN-γ, TNF-α, and IL-6 [35]. T cells are the main effector cells in cellular immunity, and $CD4^+$ helper T (T_h) cells are one of the important subgroups of T cells. $CD4^+$ T cells can be further subdivided into Th1, Th2, T regulatory (T_{reg}) and Th17 subgroups, all of which are crucial for the antitumor immunity. Th1 cells are distinguished by the secretion of IFN-γ, while Th2 cells produce IL-4. Th17 cells, a relatively recent subset of $CD4^+$ T cells, play notable roles in autoimmune diseases, infectious diseases, cancer, and transplantation by secreting IL-17 and IL-22 [36,37]. Upon recruitment to a specific site, the initial $CD4^+$ T cells undergo differentiation into Th1 cells in the presence of IL-12 and IFN-γ. Subsequently, these Th1 cells release additional IFN-γ, regulating the cellular immune response, which is closely associated with anti-tumor and antiviral effects [38]. $CD4^+$ T cells can also differentiate into T_h2 cells in the presence of IL-4, and secrete IL-4, IL-6, and IL-10, thereby promoting an antibody-mediated humoral immune response [39].

In present study, it was found that the serum concentrations of IL-17 and IFN-γ were significantly elevated in the gastric lesions model group, which may play an important role in the cellular immunity response when gastric lesions develop. Notably, the serum concentration of IL-17 and IFN-γ decreased in the MSCs transplant group, while serum concentrations of IL-4 and IL-10 increased. IL-17 and IFN-γ mainly act as pro-inflammatory cytokines that can promote the activation of T cells and stimulate the production of a variety of cytokines, while levels of IL-4 and IL-10 may reflect the humoral immune response. Transplantation of MSCs could alleviate and reverse the progression of gastric lesions by regulating immune response and levels of inflammation through decreasing the levels of inflammatory cytokines.

5. Conclusions

In summary, MSC transplantation significantly attenuated the inflammatory response in a rat model of gastric lesions. Both morphological and histological observations revealed that MSC transplantation could contribute to the recovery of gastric lesions. Transplantation not only reverses lesions and diminishes inflammation, but also holds promise for potential therapeutic effects on gastric diseases. BMMSCs may be ideal cells for stem cell therapy to inhibit the progression of gastric lesions by anti-inflammatory and immunomodulatory mechanisms.

6. Limitation of the Study

Firstly, the occurrence and development of tumors are protracted processes. Due to constraints in experimental duration, our observation of BMMSC colonization was limited to a relatively short period. Consequently, we were unable to scrutinize the enduring consequences of the interaction between the local microenvironment and BMMSCs over the long term. Secondly, the mechanism by which BMMSCs intervene in gastric precancerous lesions warrants further research.

Abbreviations

GC, gastric cancer; MSCs, mesenchymal stem cells; BMMSCs, bone marrow-derived mesenchymal stem cells; BM, bone marrow; UC, ulcerative colitis; DMEM, Dulbecco's modified Eagle's medium; MNNG, N-methyl-N′-nitro-N-nitrosoguanidine; CK, cytokeratin.

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions

QC: Original draft, Formal analysis; CW: Writing — review & editing, Data curation; WW: Resources, Software; YG: Conceptualization, Methodology, Supervision; HC: Conceptualization. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All experiments were approved by the Ethics Committee of the Animal Facility of Chinese PLA General Hospital, and were conducted in conformance with the guidelines for caring for laboratory animals.

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Conflict of Interest

The authors declare no conflict of interest.

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