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Bone marrow mesenchymal stem cells ameliorate colitis-associated tumorigenesis in mice

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ABSTRACT

Background and Aims: Bone marrow-derived mesenchymal stem cell (MSC) is widely studied in inflammatory bowel disease (IBD) in basic and clinical research. However, patients with IBD have higher risk of developing colorectal cancer and MSC has dual effect on tumorigenesis. This study aims to evaluate the role of MSC on tumorigenesis of IBD.
Methods: MSCs were isolated from the bone marrow of allogenic mice and identified by flow cytometry. Mice in the model of colitis-associated tumorigenesis induced by azoxymethane and dextran sulfate sodium were injected with MSCs. Colon length, spleen size and tumors formation were assessed macroscopically. Pro-inflammatory cytokines and STAT3 phosphorylation in colon tissues were analyzed.
Results: MSCs ameliorated the severity of colitis associated tumorigenesis compared with PBS control, with attenuated weight loss, longer colons and smaller spleens. Tumor number and tumor load were significantly less in the MSC group while tumor size remained comparable. Histological assessment indicated MSCs could reduce histological damage of the colon tissue. Decreased expression of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6), and down-regulation of STAT3 phosphorylation in colon tissue were found after MSC treatment.
Conclusion: MSCs might ameliorate the tumorigenesis of inflammatory bowel disease by suppression of expression of pro-inflammatory cytokines and STAT3 activation.

1. Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease is a chronic, idiopathic, relapsing form of inflammatory disorder in the digestive tract [1]. Chronic inflammation is believed to have decisive roles in the pathogenesis of cancer [2,3]. The shift from IBD to colorectal cancer (CRC) is one salient example of the link between chronic inflammation and tumorigenesis, which is characterized by an "inflammation-dysplasia-cancer" sequence [4]. As the most serious complication of IBD, CRC tends to develop in such patients, with a significantly increased risk compared with general population. Eaden et al. found cumulative risks of CRC in patients with ulcerative colitis were 2%, 8%, 18% after 10, 20 and 30 years of disease duration, respectively [5]; while Jess et al. showed standardized incidence ratio of 1.9 for CRC in Crohn's disease [6].

Mesenchymal stem cells (MSCs) are non-hematopoietic stem cells with the capacity of self-renewal and pluripotent differentiation into osteogenic, chondrogenic and adipogenic lineages [7,8]. Moreover, MSCs display profound immunomodulatory especially immunosuppressive properties by inhibiting proliferation and function of several major immune cells such as T and B lymphocytes, dendritic cells and natural killer cells [9,10]. Due to all these capacities, MSCs become promising therapeutic candidate in tissue engineering, regenerative medicine and autoimmune disease. Actually, MSCs have been widely studied and even tried clinically in cartilage and meniscus repair, myocardial infarcts, graft-versus-host disease and IBD [11,12]. For instance, in a randomized controlled trial conducted by Tan et al. [13], MSC treatment after renal transplantation resulted in lower incidence of acute rejection, decreased risk of opportunistic infection and better estimated renal function. In a prospective, randomized controlled clinical trial by Wong et al. [14], intra-articular injection of MSCs was proved effective in patients undergoing high tibial osteotomy and microfracture for varus knees with cartilage defects. Previously, we also discovered the anti-inflammatory effect of allogenic bone marrow-derived MSCs in an experimental mouse colitis model [15].

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However, the role of MSCs in tumorigenesis and tumor growth remains controversial. In some certain kinds of tumors, MSCs could suppress tumor growth both in vitro and in vivo, such as hepatoma, lung cancer and gliomas [16–18]. On contrary, MSCs intervention could serve tumor-promoting functions in a wide range of cancer models, such as breast cancer, prostate cancer and colon cancer [19,20]. Notably particularly, all the tumor models mentioned above were mainly performed by injecting cancer cell lines into immunodeficient nude mice. Such models may be defective as tumors can be induced by chronic inflammation [2,3].

Knowledge about what role MSCs play on tumor initiation remains limited. In this study, we aim to investigate the role of MSCs on tumorigenesis of IBD in a mouse model.

2. Materials and methods

2.1. Mice

Female C57BL/6 mice were obtained from the Laboratory Animal Center of Sun Yat-sen University, Guangzhou, China. Mice were kept in special pathogen free facility with free access to drinking water and a pellet-based diet and were quarantined for 7 days before experiment. The experimental protocol was approved by the Ethical Committee of Sun Yat-sen University. All animal studies were conducted with the approval of the Institutional Animal Care and Use Committee of Sun Yat-sen University.

2.2. Culture and identification of MSCs

MSCs were isolated from 3–4 weeks old female C57BL/6 mice as described previously [21]. Briefly, mice were killed by cervical dislocation and hind limbs were excised carefully. After removing the muscle and connective tissue from the femurs and tibias, bone marrow was flushed out from the marrow cavity with complete culture medium consisting of Dulbecco's modified Eagle's medium (DMEM, Gibco, New York, USA), 10% fetal bovine serum (FBS, Gibco, New York, USA) and 1% penicillin/streptomycin (Gibco, New York, USA), using a 0.45 mm syringe needle until the bones became pale. Cell suspension was filtered through a 70 mm filter mesh and then centrifuged at 600*g* for 3 min. After removal of the supernatant, cells were resuspended and viability and yield of cells were determined by Trypan blue exclusion and counting on a hemocytometer. Cells were then cultured in plastic culture dishes (Nest, Shanghai, China) at a density of 25×10^6 cells per ml in complete culture medium mentioned above. Medium was regularly replaced every 2 days to remove the non-adherent cells. When the culture reached over 80%, cells were digested with 0.25% trypsin for 2 min at room temperature and subcultured. For all experiments, cells at passage 3 or 4 were used.

In order to identify the cultured MSCs achieved above, we performed flow cytometric analysis. Briefly, cells were freshly retrieved after digestion and washed with cold PBS. Then cells were incubated with phycoerythrin (PE) conjugated anti-mouse Sca1, CD11b, CD34, CD 44, CD 45 and CD 105 (BD Biosciences, New Jersey, USA) in dark at 4 °C for 30 min. After washing with phosphate buffer saline (PBS) twice, cells were resuspended and examined using flow cytometer (BD FACSCanto™, BD Biosciences, New Jersey, USA). A total of 10,000 viable events were collected and analyzed.

2.3. Animal model induction and treatment

Eighteen female C57BL/6 mice (aged 6–8 weeks old, about 20 g) were divided into 3 groups (6 mice per group), including the experimental group (MSC group, receiving AOM/DSS modeling and MSC treatment), the control group (PBS group, receiving AOM/DSS modeling and PBS treatment) and the negative control group (NC group, receiving no AOM/DSS modeling or MSC).

Azoxymethane (AOM, Sigma–Aldrich, Saint Louis, USA) and dextran sulfate sodium (DSS, 36–50 kDa, MP Biomedical, California, USA) were used to induced colitis associated cancer in mice [22]. Briefly, mice were injected intraperitoneally with a single dose (10 mg/kg) of AMO, followed by 3 cycles of DSS, with each cycle consisting of 1 week of 2% DSS in the drinking water and 2 weeks of normal drinking water. On days 4, 14 and 24, mice in the MSC group were injected with MSCs (10^6 cells in 0.3 ml PBS) via the tail vein. Instead, mice in the PBS group received 0.3 ml PBS without MSCs.

Mice were monitored twice one week for the body weight, stool consistency and the presence of blood in the excreta. At the end of week 12, mice were sacrificed by cervical dislocation. Colon length (from the ileocecal junction to the anal verge) and spleen size were measured. Then colon was incised longitudinally and macroscopic tumors were counted and measured with a caliper. Segments of the distal colon were fixed in 10% neutral buffered formalin for subsequent paraffine embedding, or kept in RNA stabilization solution (RNA later, Ambion, California, USA) as tissue sample for further analysis.

2.4. Histopathological evaluation

Four micrometer-thick sections of formalin-fixed paraffin-embedded tissues were stained with hematoxylin and eosin (HE) to evaluate the inflammation severity. Colitis was scored in a blind fashion as previously published, with a combined score for tissue injury (score, 0–3) and infiltration of inflammatory cells (score, 0–3) [23]. Briefly, for tissue injury, 0 = normal colonic mucosa; 1 = discrete lymphoepithelial lesions; 2 = surface mucosal erosion or focal ulceration; 3 = extensive mucosal damage and extension into deeper layers. And for infiltration of inflammatory cells, 0 = occasional presentation of inflammatory cells in the lamina propria; 1 = increasing number of inflammatory cells in the lamina propria; 2 = inflammatory cells extending into the submucosa; 3 = transmural extension of the infiltration. The histological score was defined as the sum of the two parameters above (from 0 to 6).

2.5. Quantitative Real-time Polymerase Chain Reaction (PCR)

Total RNA was extracted from colon segments using trizol reagent (Ambion, California, USA) and then quality and concentration were assessed. RNA (1 μg) was then reverse transcribed using the ReverTra Ace qPCR RT kit (FSQ-101, Toyobo, Osaka, Japan) according to the manufacturer's protocol. Quantitative Real-time PCR was performed with SYBR Green Realtime PCR Master Mix (QPK-201, Toyobo, Osaka, Japan) on Applied Biosystems 7500 Real-time PCR system (Applied Biosystems, California, USA). Tumor necrosis factor (TNF)-α, interferon (IL)-1β, IL-6 were measured. All reactions were performed in triplicate, with 4 samples from different groups. The quantification of target mRNA was normalized by glyceraldehydes phosphate dehydrogenase (GAPDH), an internal control gene. The relative expression of mRNA was calculated by $2^{-\Delta\Delta Ct}$. Primer sequences were as follows, AGCACAG AAAGCATGATCCG (forward primer of TNF-α), CTGATGAGAGGG AGGCCATT (reverse primer of TNF-α); ACCTGCTGGTGTGTGACGTT (forward primer of IL-1β), TCGTTGCTTGGTTCTCCTTG (reverse primer of IL-1β); GAGGATACCACTCCCAACAGACC (forward primer of IL-6), AAGTGCATCATCGTTGTTCATACA (reverse primer of IL-6); TCAATGAAGGGGTCGTTGAT (forward primer of GAPDH), CGTCCCG TAGACAAAATGGT (reverse primer of GAPDH).